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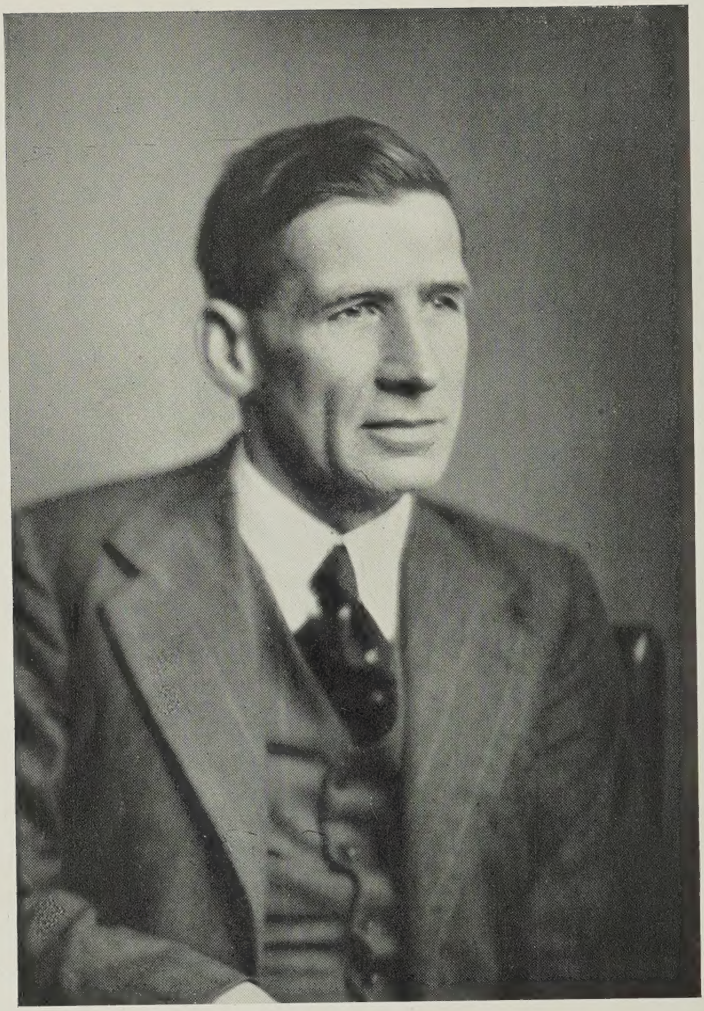

PROF. W. BROWN, F.R.S.

President of the Association of Applied Biologists, 1942–1944

Plant pathology: teaching and research

BY WILLIAM BROWN, F.R.S., *Imperial College of Science and Technology, London*

Address of the President of the Association of Applied Biologists delivered to the Annual General Meeting on Friday, 23 February 1945

(With Frontispiece)

Some time ago, when I had more or less decided to address you on the subject of plant pathological teaching and research, I proceeded to do what a supervisor of research recommends to his students as the correct procedure in the circumstances, namely, to look up the literature. I read, not widely, but selectively—which perhaps is not so correct a thing to do. To be exact, I looked back through the Addresses of my predecessors in this Office and read some of them, together with a number of papers or reports of discussions which have appeared in the *Annals* during the past 20 years. This was rather limited reading but it was enough to bring me face to face with my first difficulty, which was this, that the subject seemed to have been so fully discussed as to make it very unlikely that I could add much to it. I refer in particular to the Presidential Address given by Prof. Brierley in 1934, and to a discussion arranged by Sections K and M of the British Association at Leeds in 1927. Nevertheless, I decided to carry on, partly because fully 10 years have elapsed since then, so that conditions are now altered in certain respects, and partly because, even if certain statements have already been made, it may be a good thing to make them again. Perhaps I may be able to discuss the subject from a slightly different angle from that of my predecessors, and thus contribute something towards filling in the picture.

I should also mention a second difficulty—one which has arisen from more recent events. During the past year, a Committee of this Association has been labouring to produce a Report on the training of plant pathologists in this country—what it has been in the past and what we consider it should be in the future. It happens that I am a member of that Committee. You will agree that, in choosing a topic as I have done, I run a risk of stealing some at least of the thunder of that Committee. On the other hand, I might be allowed to recover some part of it as being my own particular thunder. If I must furnish an excuse, it is that I shall cover the field in a manner different from what has been adopted in the Report.

In his Address of two years ago, Dr Hubert Martin dealt in part with some war-time biological problems. We are now, we hope, appreciably nearer the realization of peace and of that kind of atmosphere in which biological work, and more especially research, can hope to flourish once more. We have every reason for believing that the next few years will see many changes—changes in our educational system at all levels, with resultant effects upon students who take up an applied biological profession, and changes in our agriculture which will lead to a different emphasis upon instruction and research in biological subjects. With these changes impending, it may therefore be opportune at this time to look back over the years of one's experience and see how far the old system appeared to be satisfactory and where, on the other hand, it did not give the results which might have been expected.

I have used the words 'plant pathology' in the title of this address, and I am conscious of the fact that many members of the Association will immediately feel uneasy at mention of it. These will include all members of the Council, and some others who have served on Committees. I must first of all explain what I mean by it.

My own experience has been substantially in the region of mycological plant pathology, that is, I am a plant pathologist in the narrower sense which is current in this country, as, for instance, in the Calendars of Universities and Agricultural Colleges. I do not propose to weary you with any detailed examination of this matter but shall merely say that the narrower definition leads to difficulties in practice, for example, in delineating the respective spheres of the plant pathologist and the agricultural entomologist in the study of insect-borne diseases. This is just what one has learned to expect in a subject like biology, where hard and fast borderlines are rarely discernible. Therefore I am in general agreement with those who meet the borderline type of problem by abolishing borderlines as far as possible, and this is one such case. It is much better, I think, to use the words 'plant pathology' in a wider sense, and to mean by it the study of the plant in disease, whatever the cause may happen to be. Within this wider region of study there will, of course, require to be some specialization which will be reflected in the type of training considered adequate for the various practitioners.

On this topic of definition, I would venture to go even further towards generality. The word 'disease' has come to acquire rather a special meaning, and to most people implies more than mere 'unsatisfactoriness'. I would be prepared to stake a claim as follows. Wherever a plant (or a plant product) is unsatisfactory for any reason whatever and in any respect whatever, as, for instance, when the yield has been smaller in quantity or less desirable in quality than might reasonably have been expected, there one sees a problem for the plant pathologist. No doubt it would be very easy for some of you to think out problems which fall within this widest scope and which would sound curiously if labelled 'plant pathological'. Such matters appeal to me as details, and the important point is to recognize the unity of aim, which is to achieve more and more control over the plant in certain desired directions. The technicians employed must of necessity be various—entomologists, geneticists, mycologists, soil chemists and others—but they are all harnessed to the one major purpose, the production of more and better plants. I need hardly add that this is the main interest of the public, who supply in the long run the funds which render our work possible.

The limitations of my own experience will restrict me to some extent as regards my illustrative material, and obviously I can be expected to speak with more intimate knowledge of the narrower field of mycological plant pathology than of the wider field suggested above. It will be difficult for me to avoid being to a certain extent autobiographical, but I shall do my best to keep that side under. In so far as I come to certain conclusions with regard to my branch of the subject, and granting that you are in moderate agreement, I hope that you will be able to turn them over in your minds and see how far they apply to the various studies with which you are concerned. I hope that, if I have occasion to make strictures, you will not merely say 'He is speaking of himself and his own subject', whereas if I happen to be eulogistic you will say 'Now he is thinking of us'.

I have put down in my title the phrase 'teaching and research'. The two are not easily separable, for there is general agreement that teaching will not long be very live if the research side is dead. The necessity for research in a teaching institution, quite apart from the desirability of keeping the name of the place upon the map, is all the more compelling if you agree with me that the object of teaching is not so much to inculcate a mass of facts (or alleged facts) which may soon be forgotten, as to instil methods of handling facts, and where possible of discovering new ones. In this connexion the proverb about example and precept strongly applies.

It would take too long to go into any detail as to what I think is the most desirable pre-graduation training for a man who intends to take up plant pathology as a career. While I am painfully conscious of the fact that all of us modern plant pathologists have worked into our subject in a manner wonderful to look back upon, yet I would be very loth to try to lay down any hard and fast lines. I rather think of students as I think of plants. Some are so delicate that you can hardly keep them alive; others, on the contrary, take a lot of killing. That is to say, a student with the proper mentality and purposefulness will come to fruition in spite of much mishandling by his teachers. The old system, or rather lack of system, under which we have been brought into the subject, can at least claim that it functioned in the best tradition of natural selection. But clearly one must set some limits to this sort of thing in the interests of efficiency and the saving of time. I would like to make some general observations under this heading.

First of all, what type of man, from the secondary schools, should we welcome as entrants into the biological field? Here I am giving my personal opinion, though I know that many of my colleagues are in agreement with me on this point. We must look here at the kind of training given in the schools.

There was a time, not so long ago, when botany was the only biological subject which found a place in the school curriculum. This applied only to a proportion of schools, and in these it was not on a level with the physical sciences. It was in general a subject for girls and for the weaker of the boys. In the ten years or so before the war there was a considerable change-over from botany to the composite subject biology. This movement was progressive and extended to schools which had not hitherto offered instruction in any biological subject. The expectation is that this development will continue after the war, so that we can look to a not-distant date when biology will take its place as a standard subject in schools, much on a level with physics or chemistry. From the point of view of equipping the ordinary citizen for his life's work, this change has a great deal to recommend it, and I have not met anyone who seriously contests its merits. We are concerned here with the more limited question as to how it affects the equipment of students who come up to the Universities bent upon a career in biology.

Frankly, I would say that we would rather that entrants into University Biological Departments did not have this preliminary biological training at school, and that for two reasons. One of these is probably temporary, the other I fear is permanent. As regards the first, we are still in the transition period, which may last for some time. For reasons which are sufficiently obvious, the standard of instruction at present reached varies very greatly from school to school, so that in the mixed batches who come up to the Universities it is difficult to know where best we should begin. It is particularly with regard to laboratory instruction that we find the greatest unevenness, and obviously that is very important. No doubt this difficulty will lessen as time goes on. The second

objection, of a more lasting nature, is this. Biological instruction in the schools has been developed at the expense of the physical sciences, and there is a grave risk that the biological student of the future will come up with an inferior grounding in these fundamental subjects. Once he has started on his biological work, he may never have the opportunity or the will power to go back and make good the omission, and so the field of his usefulness may be permanently narrowed. Speaking broadly, one can say that there are some subjects of a kind that if one neglects them in one's youth, one does not easily pick them up later. Physics, and more especially mathematics, are such subjects.

It is not so much the factual mass which students carry forward from their chemical or physical upbringing to which I attach importance; still less do I think that the value of mathematics lies in helping forward what in my opinion is an overdone tendency, viz. the attempt to dress up biological conclusions in the strait waistcoat of mathematical formulae. The important thing to be gained is the habit of precise thinking, which comes more naturally to those precise subjects. It is easier to draw sound conclusions from precise data than from data to which a considerable margin of error attaches, and so it is logical to step up to the biological argument by way of the chemical-physical-mathematical argument. *A propos* of this topic, I should like to tell you that, in the Institution to which I belong, we biologists are spoken of as the 'Woolly Departments'. I hope that that description refers merely to the nature of our data, and not to the uses to which we put them.

Summarizing what I have just said, I would picture the desirable type of student somewhat as follows. It is immaterial whether he has had any biological instruction or not, but I want him to be well founded in the basal sciences. I also want him to have a natural interest in plants or animals, an interest which may have shown itself already, or if not, which is readily awakened and is genuine. It follows that he should be land-minded. *A priori*, I should like him to be a countryman who preferred the country to the town, as he would probably feel more at home there and have a broad country background. Nevertheless, I confess that most of the students who have passed through my hands have been town bred, and though I have at times thought that some of them were rather raw townsmen to begin with, yet they have developed the proper spirit in due course.

Assuming now a suitable type of student, where and how are we to train him? An institution in a rural setting is an attractive picture. Situated as I am, I speak on this topic, if not with feeling, at least with a certain amount of repining. It is not merely that my Department is well buried within the limits of a very large town; it is that we are much overshadowed by the harder sciences, if I may call them so. I am not very obviously in my spiritual home. Nevertheless, there are compensations, as I shall point out.

The choice for a student lies between the Biological Departments of a University, which nearly always is situated in a large town, and an Agricultural or Horticultural College, which is usually in the country. Both have their merits and limitations, which I shall proceed to consider, though it would be difficult for me to claim that I am unbiased in this matter.

Students at an Agricultural College have the advantage that they are not brought up in a theoretical or academic atmosphere; they are in country surroundings and they are in contact to some extent with the practical side of things. At the same time I am not prepared to allow that a few years' or even a life-time's residence at an Agricultural College necessarily imparts the true inwardness of the farming life. Many farmers draw a sharp distinction between what is done at an Agricultural College and what they consider to be practicable on the average farm. From the technical point of view the great objection to the ordinary Agricultural College as a place for the training of specialists in the plant industry is the meagreness of instruction in the basal sciences. A sound and comprehensive course in the biological sciences seems to me to be essential, and that is what the Agricultural Colleges are not at present in the position to give. Of course, one must allow for exceptional cases, and I know personally some excellent research workers who have graduated by this channel. Nevertheless, I consider them to be exceptional cases.

The strength of the University Department is the weakness of the Agricultural College, and vice versa. I need not dilate upon the social advantages which accompany the University life, but shall confine myself to the technical. It is of the greatest value to a student, in his undergraduate years and perhaps more so during a period of post-graduate research, to be able to rub shoulders with workers in other scientific subjects or in University subjects generally, with consequent broadening of his outlook. There are also the special advantages of access to advanced courses on topics related to his main study, e.g. courses on physical chemistry, spectroscopy, and so on. These advantages are obvious and need no further emphasis; the disadvantages require fuller treatment.

The question has been seriously asked—Is the rarefied atmosphere of a University Biological Department a suitable training ground for a man who is going to specialize in problems of plant culture? Prof. Brierley in his Presidential Address discussed this point in a very lively manner, and had much to say about the academic man sicklied o'er with the academic cast of thought. With much of what he said I find that I am in hearty agreement, though I am optimistic enough to believe that there has been an improvement in the interval. I do not think that you will find so many of the academic die-hards flourish-

ing in University circles as you would have found 20 years ago. It is also true that courses in botany and zoology are brought into line with current interests and activities, by the introduction of new and the scrapping of old material, though it may well be that the process of modernization is not going on fast enough.

Speaking in particular of botanical courses, I should say that the greatest weakness is the lack of intimate contact with growing plants. By intimate contact I do not mean merely looking at living plants; I mean actually handling them and growing them. My own experience, as an undergraduate, is perhaps worth repeating. Though I had the good fortune to learn my botany in a Department which was housed in one of the best Botanic Gardens, I was never asked nor expected to grow a single plant, nor did I ever grow a fungus in culture. It might be urged that I was training as a botanist and not as a gardener. Quite so, but it is difficult to justify a botanical training which would inevitably make me look a fool in the presence of a practical man.

The morphological bias which has ruled so long in botany is responsible for that most familiar impression of a botanical laboratory, viz. the pervading smell of methylated spirit and the rows upon rows of specimens in pickle jars. Plant physiology, ecology and more lately genetics have helped to bring in some fresh air, but we still have some distance to travel along that line. In most institutions the facilities for studying plants in the fresh are not too good, but we hope for great improvements in the not distant future. As regards my own circumstances, I consider that I have been more than usually fortunate in the matter of laboratory accommodation and equipment, with the result that we have been able to teach mycology from the cultural-physiological point of view to a degree that is not usually attempted. As regards the handling of green plants, conditions have not been so easy, but we have made a beginning. It is no longer possible for a student to graduate in botany from my department and to be entirely ignorant of the growing of plants. I say this in presence of some ex-students of mine, that when in their final year they have been given some experiments to carry out, with a packet of seed and a plot of ground or some flower pots, their first efforts were sometimes astonishing. That was the crude beginning, but improvement was rapid, and a few months afterwards one could say that if, to speak metaphorically, they had not learnt as yet to swim, they knew the feel of water and had practised some of the movements. They certainly could say, for the first time in their lives, that they had carried out an experiment with growing plants from A to Z by their own unaided efforts. They have also learned to work with plants, and not with bits of plants.

I am convinced that we should do more of this sort of thing and begin to do so sooner. Only in that way will our botanical training come down to earth, and fit a student to face the practical man, as he must do especially if he is going to become a plant pathologist. Incidentally, it will help to remove that complaint which has been levelled against our students in the past—that they showed an aversion to working out in the field but tended rather to bring material into the laboratory and ruminate upon it there.

A further means towards the same end, which I have adopted for some time and which can be pushed further, is to encourage students to spend a substantial part of their vacations at Research Institutes or on farms. We cannot have too much of that sort of thing.

It appears to me, therefore, that the proper place for the undergraduate training of the plant pathologist is still the University Biological Department, but that it is incumbent upon the latter to incorporate as much as limitations of time will allow of the practical plant cultivation work of the agricultural or horticultural institute.

We come now to the training of students in research methods and to the problems of plant pathological research as they present themselves to a University teacher. Here I must draw very largely from my own particular experience.

At the Imperial College, about half of our Honours students take up research in plant physiology and half in plant pathology. It is noteworthy that in general those students who take the highest places in examination elect for plant physiology. Let me hasten to add that there have been important exceptions—both ways. There is a definite suggestion that plant physiology is the higher-browed discipline. It would appear that the best brains are required to deal with the subtleties of the plant physiological problem, and I leave it to you to state the converse case. Here we approach the well-worn question of the 'fundamental' versus the *ad hoc* type of research—a distinction which always irritates me intensely and one to which no doubt I shall return later. Whether the choice I have indicated is natural or whether it is a result of faults in our method of teaching, I do not know, but the fact is that the best of our working plant pathologists have come from the lower half of the first class and the upper half of the second class grouping in our Final results.

The standard course is that for the Ph.D. degree, namely, a minimum of two years' research work under supervision. It is fashionable (perhaps less so than formerly) to scoff at this degree, but I am in full agreement with the underlying principle. I should like to see a change but along the following lines: the M.Sc. degree to be given for the standard of work to which at present we give the Ph.D., and the title of Ph.D. to be abolished. A period of at least two years and of not more than three years is, to use the mathematical phrase, necessary and sufficient to set

a good student upon his legs as a research worker. At the beginning of this course the student is full of cut-and-dried ideas derived from text-books and has no idea of the minutiae which will confront him in a research problem. He is probably also lacking in confidence. By the time he has finished, he will probably have had an epitome of the remainder of his life from the research standpoint: he will have learnt that some things come out 'according to plan', some only after much worrying, and some not at all. He should have learnt how to lay down experiments, especially with regard to the scale necessary for obtaining a certain kind of result, how to assess the results obtained and how to put them together in an orderly and readable form. In all these respects the best of students are certain to require direction; it may take the form of jogging or of restraining. Perhaps I have laboured this point unduly, especially to an audience which is already well informed on the subject, but my aim was to establish that a course, the Ph.D. course, which should achieve and often does achieve these results is not lightly to be dismissed as being of little value.

The field of research in plant pathology which is appropriate for a University staff (as contrasted with that of a Research Institute) is no doubt fairly wide, but there are some obvious limitations, even when the facilities of laboratory and experimental grounds are all that could be desired. The subject chosen should be one which could be expected to yield a result within a period of about two years, even though the primary object is not to obtain publishable results as such but to give training in research methods. Two years is a short time for outdoor research, where the kind of work possible is often dictated by the season, and especially when, as often happens, the first season must be largely given over to exploratory work and to the assembling of suitable material. It is very nice to give the student a flying start, namely, by handing over to him the findings of some preliminary work, but this is not always possible.

When facilities for field work became available in 1930 at the Imperial College Field Station, I concentrated upon certain vegetable and ornamental crops as being most suitable for my purpose, and for a variety of reasons. The Field Station was in an area where many of these crops were grown on a large scale so that access to suitable farms and nurseries was easy; the plants are quickly grown and occupy comparatively small space; and, rather strikingly, the official Research Stations had paid comparatively little attention to the problems of the vegetable and ornamental plant grower. The number of problems which one meets with is indefinitely large, especially if regard be paid to the less widely cultivated crops, but a number of them are too time-consuming to be suitable for the work of Ph.D. candidates. Even with the more limited problems which one considers to be suitable, it is remarkable how often the services of two students working in succession are necessary before a particular piece of work is rounded off satisfactorily. Hence a number of conjoint papers, which do not represent collaboration in the usual sense, but successional collaboration. Variations of seasonal conditions are largely responsible for this lengthening out effect.

A useful plan in my experience has been to assign to each student two problems, one involving laboratory work and often academic in outlook which could be followed up at any time of the year, and the other an outdoor one of a seasonal nature. This has a certain insurance value from the point of view of results, and is particularly useful in helping to tide the student over the earlier slack and sometimes bleak phases of the work.

Teaching and research of the kind which I have been describing, helped by my natural inclination in these matters, have had the effect of bringing me into somewhat close contact with the practical grower and his problems. For these contacts I am indebted almost wholly to the good services of Mr W. Buddin of the Southern Agricultural Advisory Province, and it is with particular pleasure that I take this opportunity of recording my very great indebtedness to him. It happens also that I come of farming stock and am personally acquainted with many farmers and their ways of looking at things. I can, therefore, justly say that I have had a certain amount of experience of science as applied to agriculture at three points—at the production end, at the distributional intermediary, and at the receiving end. I may therefore be allowed to put down some of my reflexions on the functioning of the research machine, and these will fittingly conclude my address. As there will be some criticisms, I wish to make it clear that, henceforth, I shall be speaking of plant pathology in the narrow sense where my own experience lies and where I am surer of my ground. How far you accept my views and how far you are prepared to apply them to your own particular fields is a matter for you to decide.

First of all there is the much-debated question of the relative importance of academic and applied research, not merely from the point of view of research as such, but also of research as leading to results of practical value. It has always seemed to me that, in discussions on this topic, much confusion of thought has arisen from a more or less unconscious misuse of the rather numerous names which have been applied in this connexion. If we say that there are, broadly speaking, two types of research, the theoretical which aims at understanding phenomena without reference to any practical application of its findings and the practical which aims at a definite practical result, there will, I think, be general agreement that both are important and desirable, and in many cases there will be found to be an interdependence between the two. Unfortunately, it has been customary to apply

a superfluity of names to each type of research, and the use of some of these names has led to misconceptions and to the misdirection of research. Thus, the theoretical type is known as 'academic', 'fundamental', 'basal' or 'long-term', and the practical as 'applied', *ad hoc* or 'short-term'. I would like to comment upon some of these terms.

If by 'fundamental' is merely meant 'theoretical' as roughly defined above, I have no objections to it. There is, however, a strong tendency to read a further meaning into the word. Just as a house cannot be built unless its foundations or fundaments are properly laid, so, it is argued, must the practical work follow and rest upon the earlier fundamental investigation. One hears it freely stated that from such fundamental work results of practical importance drop out from time to time, and all that remains for the practical man is to recognize them as such or to seize upon certain principles, proceeding from which he can readily arrive at practical results. Now I am well aware that there is no great difficulty in furnishing examples in support of such views, more notably in certain physical arts, such as radiolocation, in which the practice has been built up upon results which a few years ago were of theoretical interest only. The relation of the biological sciences to agriculture is, however, somewhat different, as I need hardly enlarge upon. Most of the results of practical value have been obtained by the growers themselves, sometimes by accident, and in such cases the function of agricultural science has largely been to explain the results, to point out their limitations and sometimes to indicate improvements. It is well to recognize the fact that results of the very greatest practical importance have been obtained in connexion with problems, the theoretical analysis of which is quite unknown to this day. For instance, it has been possible to breed potatoes which to all intents and purposes solve completely the problem of wart disease, and yet we are still quite ignorant of the theoretical basis of this very valuable result. Similar examples will no doubt occur to you.

It must not be thought that, in speaking as I have done, I am disparaging the theoretical type of research. I should be roundly accusing myself if I did so. It is obvious that theoretical research in biology generally, and in plant pathology in particular, has the same status as it has in any other science or form of learning: that it is worthy of being carried on, and carried on vigorously, on its own account. It has cultural value and adds its quota to the progress of civilization. It tends to fill in the general scientific picture, and from the point of view of the research worker himself it helps in many cases, if I may use the phrase, to keep his soul alive. It is, however, idle to maintain that practical results cannot be obtained without theoretical research, and it has always appeared to me that it is undignified, to use a mild term, to put forward as a justification of theoretical research the possibility that results of practical value may arise from it by accident. No doubt if pursued long enough and with sufficient insight, it may point the way to a solution of all our practical problems, but that date is not yet. The practical method, I feel, must still be to tackle the practical problem with the means at one's disposal, and in so far as difficulties arise to refer these back for closer investigation in the hope that new insight may be obtained. In other words, the practical method should be that of direct attack, with theoretical research harnessed to it for purposes of guidance and further development.

The description 'long-term' as applied to theoretical research is appropriate. I should be happy to see it replaced by 'everlasting', for the more successful a piece of theoretical research proves to be, the more does it suggest further problems. On the other hand, I heartily deplore the tendency to apply the word 'short-term' to applied research. I have no objection to the phrase *ad hoc*, in so far as it means that there is a definite objective, but it is unfortunate that the adjective 'definite' is so often paraphrased to 'limited' and so to 'short-term', which in many cases has come to mean 'two years or thereabouts', the normal duration of a research scholarship. The definiteness of an objective has no obvious relation to the time required to reach it, and in biological research, especially when its results are sharply conditioned by seasonal factors, it is surprising how long a time is necessary for the proper working out of even a simple problem. If, for example, it be the matter of the use of a fungicide, even when the best form of fungicide and the mode of its application have been settled, a great deal of work remains in testing its efficiency in various situations and over the variety of conditions which arise in a succession of seasons. I do not believe that the time-consuming nature of much applied biological research has been properly appreciated by those responsible for its organization, and to this more than to anything else is to be ascribed the fact that much research has failed to reach the farmer in a form usable by him. I shall refer to this point again.

The inadequate provision for the *ad hoc* type of research is illustrated in another way. Fundamental research, from its long-term nature, and because it gives no promise of early results, is not the kind of thing which the cultivator could be expected to subsidize. It must rely therefore upon official support, and examples of reasonable support for it are familiar to all of you. On the other hand, it has often been expected that for work of the *ad hoc* type the industry concerned should pay a substantial share of the cost. This would seem on the face of it to be a reasonable arrangement, and it has in fact functioned, but there are inherent difficulties. Perhaps I can illustrate this point by an experience of my own. When an investigation of the carnation wilt problem had been in progress for two years, support for its continuation

was sought from the carnation growers. Very naturally the lead was taken by certain members whose nurseries were at the time being seriously ravaged by the disease. Others, however, were as yet untouched and therefore not so interested. It was freely hinted that the trouble might not be a bad thing in itself, as tending to prevent over-production and to weed out the inferior growers. Later, it may be added, the disease spread to other nurseries so that some growers had to change their opinions. One grower said that he knew of a cure, and another that he did not propose to subscribe but that he would pay for the cure when found. It so happened that there was present a man of very great importance in the industry who took the line that though his firm was not as yet seriously troubled by the disease, the industry as a whole was in danger, and it was highly desirable that he should have as much information as possible in advance of its coming. And so the scheme came to be supported.

I quote this example, not so much to illustrate how vexatious and chancy the initiation of research in this way may be, but to stress the fact that the financial interests of the growers of any one commodity are not necessarily identical. This is true of carnation growers, who work in the comparatively standardized conditions of glasshouse cultivation, but more so when one considers growers in different areas with substantially different climates. Think in this connexion of the relation of virus diseases to the present location of the seed-potato industry. It is almost inevitable that the control of any plant disease will lead to a dislocation of the industry, and that at least over a period some growers will be harmed by it.

Perhaps I should indicate here what, in my view, is the solution of this problem. The effect of the successful application of research to a problem of plant production is that the yield of produce of a certain quality is increased. This might lead either to a reduction in cost to the public, i.e. to a raising of the standard of living, or to a reduction in the amount of land necessary for growing the required amount of produce, an economy which is obviously desirable in a small country such as Great Britain. In either event the advantage is national, and therefore the responsibility for initiating and supporting such *ad hoc* work should rest upon national funds, even though some growers may benefit, temporarily or permanently, from the results obtained.

I am hopeful that in the near future we shall see some improvements in the organization of plant pathological research. Hitherto, except for a few cultivations such as fruit, applied research has been of a very occasional and scattered nature. There has been little co-ordination, and, so far as I can see, a lack of purposefulness in following up the 'worthwhile' problems to a stage where the results could be confidently brought to the notice of cultivators. It is the function of no one in particular to collate the work which has been done on a particular problem and to devise steps for carrying it forward to a practical solution. When such a survey is made I am afraid that it will reveal a scarcity of practical methods of proved value, for the conditions under which much research has been done have been such as to limit its scope to purely mycological phases. I am not trying to be funny when I suggest that this state of affairs is reflected in the *abundance* of control measures which figure in plant pathological text-books. I am quite certain that many of the methods put forward are of doubtful practicability or usefulness, and that very few have been worked out in a manner which would convince a practical man.

The number of problems awaiting a practical solution is so great that a rigorous selection would be necessary to allow of adequate attention being paid to those deemed most important. What we require is a 'priority list' of problems, and when such a list is drawn up, I hope that the views of research workers and growers will be sought more freely than has been the case in the past.

The practical aim of plant pathological research is not to study plant disease as such but to point the way to growing good plants in spite of a liability to disease. It seems obvious, therefore, that it should be organized on a crop basis. There must be research institutes for the main crop plants, with small and possibly movable field stations where the more local problems can be investigated on the spot. We have moved some distance in this direction, and I think there will be a fuller development before very long.

As regards the connecting link between research worker and grower, I speak mainly as a spectator. Some of you know much more of this matter than I do, but I would like to record one or two impressions. I could easily be tempted to dilate upon psychological aspects of the relation of Advisory Officer to grower: how, for instance, a grower may be suspicious and look upon the Adviser as one more instance of governmental interference or spying; another is, as near as may be, unteachable, while yet others immediately seize upon a new method and straightway overdo it, on the principle that you can't have too much of a good thing. It has always appealed to me that the mycological Adviser is in a more difficult position than his entomological brother, inasmuch as the grower is familiar with the idea of insects as pests on his plants, whereas the conception of fungi as responsible for bad plants is more difficult. I remember in this connexion the grower of lettuces who put this question: 'Tell me, does *Botrytis* ever turn into a cutworm?' He was trying to hitch the unintelligible on to the intelligible, a perfectly legitimate thing to do. This is, I think, the reason why growers do not so much mind showing you their insect-damaged plants—that sort of thing is all in the day's business—but they consider that decayed or half-dead plants are not quite respectable, and

tend to conduct you elsewhere. They also tend to look upon the Advisory Mycologist as a person with a morbid interest in decrepit plants, and therefore as something of a curiosity himself. I have, however, a more serious point to make, and with this I shall conclude.

It has often been stated that research workers have in their possession a mass of information which the growers do not put to practical use, either because it is not brought to their notice at all, or if so not in a form which they can understand. The blame for this is laid upon the advisory organization. I would like to record what has been a frequent experience of mine when visiting nurseries in the Reading area in company of the Advisory Mycologist. The object of the visit might relate to one particular problem, but by the time we had gone half-way round the nursery a dozen other queries had been put. There was obviously a multitude of problems, to many of which it was impossible to give a definite answer. Sometimes one could say that 'Somebody in America had described a thing rather similar'. The plain fact is that there are many growers' problems, of all kinds and dimensions, to which no clear answer can be given, because the necessary research work has not been done. This is a point which I have already stressed. Doubtless the advisory services could be made to function more efficiently—for example, by arranging for facilities whereby they could demonstrate the value of approved methods of culture—but I feel sure that, in the plant pathological field at least, the main lack is in the paucity of appropriate research, and not in the inability of the advisory services to convey such useful information as exists.

To sum up. There is no doubt as to the desirability and feasibility of tightening up our methods of training students for applied biological work, both in their undergraduate years and in their training as research workers, but I feel certain that the greatest requirement is a well-directed concentration upon the problems which the practical grower is prepared to put before the scientific worker. There are many such, and if, as appears to be very likely, there is to be a continued intensification of agriculture in these islands, there will be many more in the future. There is no doubt, therefore, as to the magnitude of the field, and it is a responsibility devolving upon us, as scientific workers, to show that the practical problems of plant cultivation can be solved by the methodical application of the scientific method.

The infected root-hair count for estimating the activity of *Plasmodiophora brassicae* Woron. in the soil

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(With Plates 4 and 5)

A method is described for estimating the relative numbers of *Plasmodiophora brassicae* spores germinating in different soils, by counting infected root hairs. Cabbage seedlings are grown for 1 week at 25°C. in glass tumblers of the infected soils; after washing out, the tap roots are stained in 1% aceto-carmine and a count is made of the number of root hairs containing zoosporangia of *P. brassicae*, along 2 cm. of root. It is shown that by this method it is possible to study, for example, the action of different bases in inhibiting root-hair infection; the main inhibiting factor was found to be soil alkalinity, however produced. Other factors were found to influence infection in lesser degree; thus the number of infected root hairs was reduced in soils receiving *N*/10 sodium and potassium chlorides in place of distilled water. Root-hair infection was also inhibited by low soil-moisture content.

Introduction

Investigation of the effect of soil conditions upon the incidence of soil-borne disease has long been handicapped by the difficulty of controlling the soil environment. Diseases caused by soil-borne parasites often require to develop for weeks or even months after initial infection of the host before macroscopically obvious symptoms appear. Even then, the quantitative measurement of disease is difficult; the various 'disease ratings' so far devised are not completely satisfactory, inasmuch as the figures are derived from personal judgement of disease intensity rather than from absolute measurement. No statistical treatment of the data, however elaborate, can overcome the fundamental weakness of such 'disease ratings'.

Two main difficulties have therefore to be overcome in investigating the effect of soil conditions upon the incidence of a soil-borne parasite: first,

* The initial work was done by the senior author in 1935 and 1936, before resigning from Rothamsted Experimental Station to join the Ministry of Agriculture and Fisheries.

that of maintaining uniform conditions in relatively large volumes of soil for long periods, and secondly, that of measuring disease intensity. The first difficulty has been overcome to a large extent by such devices as the Wisconsin soil temperature tanks and controlled temperature-humidity cabinets, and these are necessary when the full course of development of a disease has to be studied. The second has been only partially met by the use of disease ratings.

For certain diseases in which environmental effects during a limited developmental phase are significant, it is possible to *evade* some of the difficulties of long-period control of the environment by growing plants in small volumes of soil for short periods only. It is then necessary to use a microscopical method for estimating intensity of infection. At least in some instances this has the advantage that it permits the making of precise measurements of the degree of infection, thus overcoming the second difficulty mentioned above.

For this short-period method of study, glass tumblers, holding 200–300 g. of dry soil, are used as soil containers. The use of glass tumblers as soil containers is not a new one; they were employed by Briggs & Shantz (1912) in their studies of the wilting coefficient of different soils, and by Conn (1932) in observations upon the soil microflora by means of the Rossi-Cholodny slide technique. The tumbler method was first applied to the study of a root-infecting fungus by Garrett (1934, 1936), who used it to measure the rate of growth of *Ophiobolus graminis* Sacc. along the roots of wheat seedlings. Good correlation was obtained between the growth rate of the fungus and the known incidence of take-all in the field under different soil conditions.

A study of the early stages of infection of cruciferous seedlings by *Plasmodiophora brassicae* Woron. suggested that intensity of infection could be measured by counting the number of infected root hairs on the tap roots of young seedlings. It was found that if cabbage seed was sown in tumblers of soil inoculated with a spore suspension of *P. brassicae* and the young seedlings were washed out after a week's growth at 25° C. and stained with aceto-carmine, then infected root hairs could be counted without difficulty (Pl. 4, *w*). This period of incubation permits full development of zoosporangia in the majority of infected root hairs. The development of these zoosporangia from plasmodia in infected root hairs was first described by Cook & Schwartz (1930), and their discovery was confirmed by Fedorintschik (1936). It is often overlooked that the plasmodia and sporangia in the root hairs were figured by Woronin as long ago as 1878. Woronin, however, failed to recognize the zoosporangial character of this phase of the life history, which was not correctly interpreted until it was studied by Ivimey Cook & Schwartz. A full account of this and other stages in the development of *P. brassicae* in the host is to be found in Karling's (1942) monograph of the Plasmodiophorales (p. 22 et seq.). As published illustrations of zoosporangial formation and discharge are few and not very accessible, a selection of the drawings and photographs made during the course of this work is given in Pls. 4 and 5.*

The method

The handling of soils and filling of tumblers was carried out in the manner described by Garrett (1938). The spore suspension of *P. brassicae* was prepared by grinding in a mortar a small portion of a clubroot gall that had been kept frozen. Galls that had been frozen were much easier to grind than fresh galls. The suspension of spores was freed from crushed cell tissue by straining through organdie muslin, and diluted to a concentration permitting of easy counting in a haemocytometer. In the earlier experiments, aliquots of spore suspension were added to individual planting holes made in the soil with a glass rod; later, it was found better to add the spore suspension in the whole of the water (together with any substances to be tested in solution) to the air-dry soil. After inoculation, tumblers were left for some 24 hr. on the laboratory bench (mean temperature 15–20° C.); twelve presoaked cabbage seeds were then planted in each tumbler and covered with 0·25 cm. of soil, and all tumblers were covered with glass lids and placed in the incubator at 25° C. for 1 week.

After washing out, the five strongest seedlings (i.e. those with the longest tap roots) were selected from each tumbler, and dropped into specimen tubes containing 1 % aceto-carmine. The seedling roots could be kept for several months in this stain without detriment to the subsequent procedure of counting infected root hairs. The zoosporangia at first stained a carmine pink, but the colour deepened with length of immersion in the stain. At the end of 1 week most of the sporangial groups were fully differentiated, but not yet discharged. In this condition they took the stain readily and counting was easy. If washing out was delayed much beyond a week, some sporangial groups were often found to have discharged their zoospores; and as the empty sporangia did not stain well, delayed washing out tended to give lower counts.

Infection takes place over a brief period only, when the root hairs are young and full of protoplasm. This was well shown in an experiment in which two sets of tumblers, one containing 4-day-old seedlings and the other containing freshly planted seeds, were

* Since this paper was completed G. W. Ayers has published observations on infection of the root hairs of cruciferous seedlings by *P. brassicae* (*Canad. J. Res.* Sec. C, **22**, 143, 1944). Our observations agree with those of Ayers except that we have found that zoospores are sometimes discharged into the cavity of the root hair, as figured in Pl. 4.

inoculated by pipetting into the planting holes equal volumes of spore suspension containing 1·5 million spores per ml. No infection occurred on the first 2 cm. of root of the 4-day-old seedlings, on which the root hairs were already formed and vacuolated at the time of inoculation, whereas over 400 root hairs per seedling were infected on the first 2 cm. of root of the plants grown from seed sown at the time of inoculation. In the latter case, of course, the young roots had to grow through the inoculated zone of soil a day or two after the spores had been added, so that the root hairs would have been exposed to infection from the time of their initiation.

For the counting of infected root hairs the seedling tap roots were laid out, under a long cover-glass, on a microscope slide ruled at 1 mm. intervals; this device simplified the task of counting. The counting was done under a 1 in. objective with oculars of 10× magnification; a mechanical stage was found to be essential. The number of root hairs containing zoosporangia was counted along 2 cm. of the seedling tap root; the infected root hairs on one side of the root were counted, then those on the other, and the two totals added. Infected root hairs lying above and beneath the semi-opaque mass of the root itself were ignored; difficulties of counting were reduced by this convention, and it is considered nothing was lost by it as the figures desired were the *relative* numbers of infected hairs on comparable portions of roots. Infected root hairs on lateral rootlets were also ignored. In one experiment (Exp. II) the count was made along the first and second centimetre of tap root from the seed, but in all other experiments the count was made along the second and third centimetre from the seed. The first centimetre of root was ignored because it was thought that conditions in the surface layer might not be representative of those obtaining in the main bulk of the soil.

An indication of the variation in the counts between the different seedlings in a tumbler and between different tumblers treated similarly is given by the figures in Table 1, which refer to fifteen seedlings in three tumblers, comprising a single experimental series. This series was chosen at random for illustration, and the degree of variation is typical of that in most experiments.

Table 1. *Number of infected root hairs per 2 cm. root*

No. of seedling...	1st	2nd	3rd	4th	5th	Mean
Tumbler no. 1	312	123	217	334	218	241
Tumbler no. 2	252	176	352	354	373	301
Tumbler no. 3	625	180	501	89	154	310
						284 ± 38

The standard error of the mean for any series can be calculated by the usual method, and the significance of differences between means can then be estimated by the *t* test.

In most of the experiments reported in this paper the differences between treatments are so great that they are obviously significant. For the sake of clarity therefore, standard errors have been omitted from the tables. The significance of the smaller differences is referred to in the text where necessary.

Experimental

Exp. I. In this experiment, the effect of varying density of spore suspension upon root-hair infection was studied in three types of soil at a moisture content of 80% saturation; each soil was a mixture of 90% by weight of quartz sand with 10% of a loam soil. The *p*H values of the three sand-soil mixtures were 5·8, 6·2 and 7·7 respectively. Results of the experiment are given in Table 2; the figures are means derived from 4–8 plants per series.

The number of infected root hairs increased with rise in density of the spore suspension, as was to be expected; it was also greatest in the soil at *p*H 6·2 and least in that at *p*H 7·7. A point of interest is that whereas infection was completely inhibited at the three lowest spore concentrations in the alkaline soil, it did occur at the two highest spore concentrations. This suggests that some of the reported instances of the failure of lime to control club-root may perhaps be explainable as due to exceptionally high spore concentrations in the soils concerned.

Table 2. *Number of infected root hairs per 2 cm. root*

No. spores per ml. suspension ...	10,000,000	1,000,000	100,000	10,000	1000
Soil/sand, *p*H 5·8	—	90	64	7	1
Soil/sand, *p*H 6·2	—	433	217	151	64
Soil/sand, *p*H 7·7	188	9	0	0	0

Exp. II. As lime and other alkaline substances are well known to inhibit development of club-root, an experiment was planned to determine the effect of the hydroxides, carbonates and chlorides of the alkali metals and of aluminium hydroxide upon root-hair infection. The experiment was carried out in a mixture of 90% by weight of sand with 10% of Woburn sandy loam, brought to a moisture content of 87% saturation by addition of spore suspension in *N*/100 solutions of the various hydroxides, carbonates and chlorides to be tested. As this was one of the earlier

experiments, density of the spore suspension was not estimated by a haemocytometer count, but was so adjusted that the suspension was just faintly opalescent. Results of the experiment are given in Table 3; the figures are means derived from eight plants in two tumblers per series.

In the soil receiving spore suspension in distilled water alone, the number of infected root hairs was 334 and the final *p*H value was 6·6. The results of this experiment show that root-hair infection has been almost completely inhibited by the hydroxides and carbonates of all the metals except aluminium. The chlorides, on the other hand, appear to have had little if any influence on infection, apart, perhaps, from barium chloride, which has reduced the number of infected root hairs to one-third of that in the control tumblers receiving distilled water alone. These results, taken in conjunction with the *p*H values also given in Table 3, suggest that the action of these metallic compounds upon infection operated chiefly through their effect upon soil *p*H.

Exp. III. This experiment was designed further to elucidate the effect of soil reaction upon root-hair infection. The experiment was carried out in a sand-soil mixture containing 10% by weight of an acid allotment loam; the *p*H value of the mixture was 6·3. Four bases, the hydroxides and carbonates of sodium and potassium, were prepared at $N/5$ concentration; to aliquot volumes of these bases sufficient quantities of hydrochloric acid were added to neutralize the whole volume of base, $\frac{3}{4}$ vol., $\frac{1}{2}$ vol., and $\frac{1}{4}$ vol. of base respectively. These series will be referred to as 'fully neutralized', '$\frac{3}{4}$ neutralized', '$\frac{1}{2}$ neutralized' and '$\frac{1}{4}$ neutralized'. In this way, a series of solutions containing identical concentrations of $Na^{\cdot}$ and $K^{\cdot}$, but different concentrations of $H^{\cdot}$ and OH', were prepared. To aliquot volumes of all these solutions and of the original $N/5$ bases were then added equal volumes of a spore suspension containing 2 million spores per ml. The resulting mixture thus consisted of a spore suspension of density 1 million spores per ml. suspended in $N/10$ base. Immediately after mixing, the resulting spore suspension was poured on to the soil of the three replicate tumblers comprising each series. The results of this experiment are given in Table 4; the figures are means derived from fifteen plants in three tumblers per series.

In every series, the rise in number of infected root hairs with progressive neutralization of the base, and fall in *p*H value of the soil, is very consistent. The sodium and potassium hydroxide series have influenced infection very similarly. The two carbonates have permitted a significantly higher infection than the two hydroxides at the $\frac{3}{4}$ neutralization level, and potassium carbonate has permitted significantly higher infection than any other base at the $\frac{1}{2}$ and $\frac{1}{4}$ neutralization levels (significance estimated by the *t* test). Inspection of the *p*H values of the soils at the end of the experiment suggests that these differences cannot be attributed to hydrogen-ion concentration; it was found, however, that 24 hr. after the solutions were first mixed with the soils, the *p*H values in the two hydroxide series were higher than those in the two carbonate series, so that this difference, though transient, may have persisted long enough to have influenced root-hair infection. This

TABLE 3. *Number of infected root hairs per 2 cm. root*

	Sodium	Potassium	Calcium	Barium	Magnesium	Aluminium
Hydroxides	0·3	5	0·4	4	0·1*	303
Carbonates	0	1	7	0·5	0	—
Chlorides	296	424	286	112	379	—

*p*H *values of soils at end of experiment*

	Sodium	Potassium	Calcium	Barium	Magnesium	Aluminium
Hydroxides	7·8	7·4	7·5	7·5	8·0*	6·7
Carbonates	8·1	8·0	7·2	7·8	7·9	—
Chlorides	6·6	6·6	6·4	6·3	6·4	—

* Magnesium oxide (MgO) was used in place of the hydroxide.

TABLE 4. *Number of infected root hairs per 2 cm. root*

	NaOH	KOH	Na_2CO_3	K_2CO_3
Fully neutralized	205	265	284	246
$\frac{3}{4}$ neutralized	27	25	95	141
$\frac{1}{2}$ neutralized	3	2	3	54
$\frac{1}{4}$ neutralized	0·1	0·1	0·1	19
0 neutralized	0	0	0	9

*p*H *values of soils at end of experiment*

	NaOH	KOH	Na_2CO_3	K_2CO_3
Fully neutralized	6·3	6·3	6·1	6·3
$\frac{3}{4}$ neutralized	6·7	6·7	6·7	6·7
$\frac{1}{2}$ neutralized	7·3	7·3	7·2	7·1
$\frac{1}{4}$ neutralized	7·6	7·5	7·4	7·5
0 neutralized	7·7	7·7	7·5	7·7

suggestion still leaves unexplained the consistently higher infection in the potassium carbonate series.

Infection in this experiment has evidently been influenced by other factors as well as hydrogen-ion concentration; in an additional series of three tumblers receiving distilled water alone, mean root-hair infection was 713, at *p*H 6·3. The addition of the fully neutralized bases to the soil, therefore, has reduced infection to one-third of that in the soil receiving distilled water alone, though it has not affected *p*H value of the soil. This effect of *N*/10 sodium and potassium chlorides in reducing infection recalls an analogous effect obtained by Smith & Walker (1941) when investigating root rot of peas due to *Aphanomyces euteiches* Drechsl. in nutrient sand cultures. The incidence of disease, which was severe at the 1/10 concentration of the nutrient solution, declined as the nutrient concentration increased, so that only a low percentage infection occurred at the 3 × concentration, and none at the 4 × concentration of the nutrient solution. Smith & Walker employed a suspension of *A. euteiches* zoospores as inoculum in these experiments; these zoospores and the swarm spores of *P. brassicae* (derived from germination of the resting spores) are alike in possessing no cell wall.

Exp. IV. The two preceding experiments have established a correlation between the well-known effect of soil alkalinity in inhibiting development of club-root in field and pot tests, and its effect in inhibiting root-hair infection in seedlings. The other factor that has been observed particularly to influence the development of club-root in the field is soil-moisture. The effect of high soil-moisture content in promoting development of club-root in the field has been confirmed in the glasshouse by Monteith (1924); he obtained clubbing in pots of soil held at 60% saturation and above, but not in those held at 45% saturation or below. Naoumova (1933), however, obtained clubbing in soil at 45% saturation, but not in that at 30% saturation. This discrepancy presents no serious difficulty, as soil texture is well known to affect the proportion of total soil moisture available to the roots of plants; the proportion available to soil micro-organisms must also vary with soil texture.

This experiment was therefore designed to test the effect of soil-moisture content upon root-hair infection in two soils of widely different texture. This was achieved by diluting an allotment loam, of *p*H 6·3, with sand adjusted to the same *p*H; 1 vol. of soil was mixed with 3 vol. of sand. The *p*H value of the sand-soil mixture was thus identical with that of the undiluted soil. Three soil-moisture contents, viz. 30, 55 and 80% saturation, were compared in each soil. In order to secure uniform distribution of soil moisture, each soil was thoroughly mixed by hand with a spore suspension of *P. brassicae* in sufficient distilled water to bring the soil-moisture content to 30% saturation; the two higher soil-moisture contents were then obtained by adding the requisite volumes of water to the soil surface after the tumblers had been filled. The same total number of *P. brassicae* spores (3,840,000) was thus added to the soil of each tumbler, but the density of spores in the soil water was necessarily greatest in the soils at 30% saturation; it was highest (200,000 spores per c.c.) in the sand-soil mixture, which required least water to bring it to 30% saturation. Mixing the spore suspension with soil by hand before filling the tumblers was deemed necessary in order to secure a comparable distribution of spores in the two soils; it seemed likely that if the spore suspension had been added to the surface of the dry soil, fewer spores would have passed down to the lower layers in the loam soil than in the sand-soil mixture. The results of Exp. IV are recorded in Table 5; the figures are means derived from fifteen plants in three tumblers per series.

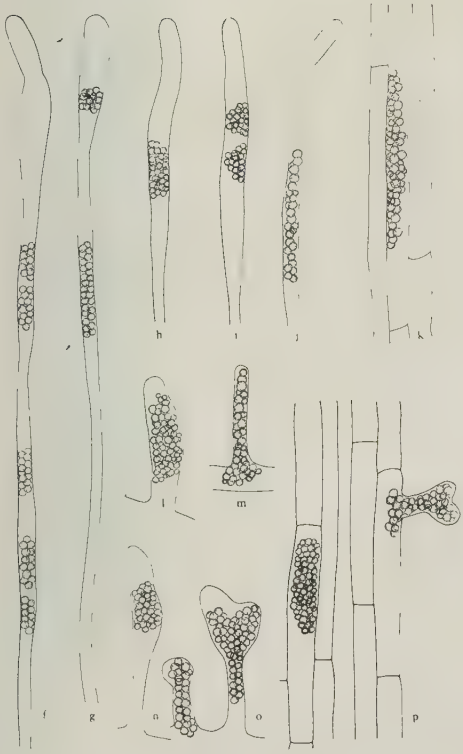
Table 5. *Number of infected root hairs per 2 cm. root*

	Moisture content of soil		
	30% sat.	55% sat.	80% sat.
Whole soil	0·1	24	340
1 soil : 3 sand	223	502	582

The number of infections increased with increasing moisture content in each soil; at every moisture content there were fewer infections in the whole soil than in the sand-soil mixture. It is interesting to note that whereas there were 223 infected root hairs in sand-soil at 30% saturation, there was only 0·1 (actually one root hair infected in fourteen plants) in whole soil at the same moisture content. Evidently the proportion of soil moisture available for spore germination and root-hair infection by *P. brassicae* varied with soil texture.

Conclusion

The method described above makes it possible to examine the initial stage of infection of *Brassica* seedlings by the club-root organism with much greater quantitative accuracy than has been possible hitherto. Obviously, much remains to be done in determining the relations between the initial root-hair infection here measured and the subsequent zoospore or zygote infection leading to growth of plasmodia in the roots and the development of 'clubs'. It may, of course, be that a very few root-hair infections can lead as surely to root infection and subsequent club formation as if hundreds of root hairs are infected. Nevertheless, there is a general correspondence between the infection trends recorded for different treatments and experience with



SAMUEL AND GARRETT—*PLASMODIOPHORA BRASSICAE* IN ROOT-HAIRS

Fig. 1

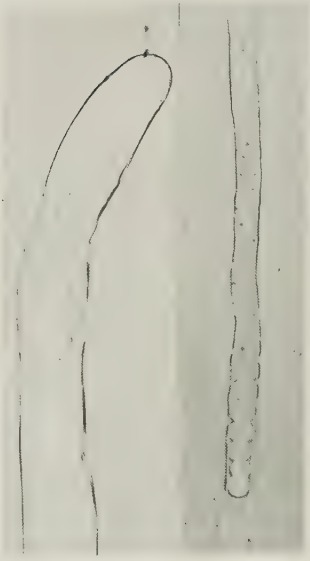


Fig. 2

Fig. 3

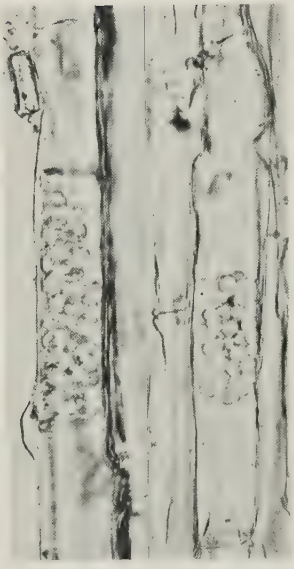


Fig. 4

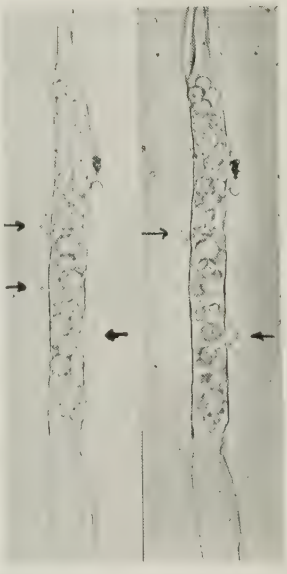


Fig. 5

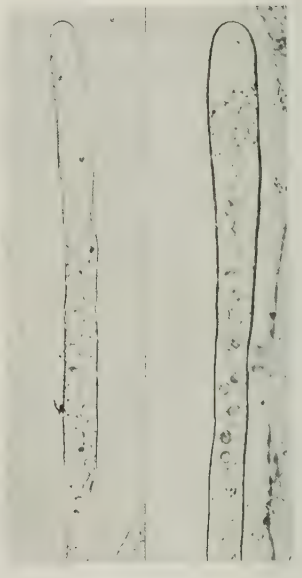


Fig. 6

SAMUEL AND GARRETT—*PLASMODIOPHORA BRASSICAE* IN ROOT-HAIRS

certain of these treatments in the field; this suggests that the gradients observed will very likely be found to be correlated with field reaction under similar conditions, even though, in particular experiments, the range of infection of root hairs may not extend down to zero.

The work does not pretend to be a full investigation of such matters as the effect of alkalinity or soil-moisture content on infection by *Plasmodiophora brassicae*. The experiments described in the paper were chosen merely as illustrations of the way in which the root-hair count method may be used to examine such questions. It remains to be seen whether use of the method will give results of value, not obtainable by the growing of plants in pots or in the field, in the investigation of particular problems concerning the club-root disease.

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EXPLANATION OF PLATES 4 AND 5

PLATE 4

Plasmodiophora brassicae in the root hairs of cabbage seedlings; drawings of stages in zoosporangial formation and discharge. *a–c*, the zoosporangial plasmodium; *d–e*, differentiation into sporangia; *f–g*, root hairs containing several sporangial groups, presumably resulting from infection by more than one swarm spore; *h–p*, root hairs with sporangial groups, showing various types of hypertrophy of the root hair, and two infected cortical cells; *q–s*, emergence of zoospores, some within the cavity of the root hair, but most escaped into the surrounding water; *t*, *u*, empty sporangia in root hairs; in *u*, refringent bodies are present which may possibly be zygotes resulting from the fusion of two zoospores; *w*, photograph of infected root hairs, in which the sporangial groups have been stained deep red with aceto-carmine. From slide prepared for counting.

PLATE 5

Plasmodiophora brassicae in the root hairs and root cortical cells of cabbage seedlings; photomicrographs showing zoosporangial formation and discharge.

Fig. 1. The zoosporangial plasmodium before differentiation into sporangia.

Fig. 2. The zoosporangial plasmodium differentiating into sporangia.

Fig. 3. Sporangial groups in root hairs.

Fig. 4. Sporangial groups in cortical cells.

Fig. 5. Zoospores escaping from sporangia, through the walls of the root hairs; two photographs of the same hair, taken at a few minutes' interval. Some zoospores visible in the first photograph had swum away into the surrounding water by the time the second was taken, whereas some further zoospores are seen to have emerged in the group on the right.

Fig. 6. Empty sporangia in root hairs. In the right-hand root hair are a few of the refringent bodies referred to in the explanation of figure *u* on Pl. 4.

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The effect of sulphanilamide and other bacteriostatic drugs on the growth of moulds

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(With Plates 6 and 7 and 7 Text-figures)

Some fifty isolates of moulds, mainly *Aspergillus* spp. and *Penicillium* spp., were tested for their reaction to sulphanilamide, sulphonamide E.O.S., sulphapyridine, sulphamezathine, sulphaguanidine, propamidine and phenamidine.

The moulds showed a high degree of specificity in their reaction to the drug concentrations: *Aspergillus* spp. were more susceptible to the drugs than were *Penicillium* spp. Most of the species included in the *Aspergillus glaucus* group were inhibited by concentrations of 1/8000 (125 p.p.m.) of sulphanilamide, sulphonamide E.O.S. or propamidine. Similar concentrations of sulphapyridine, sulphamezathine, sulphaguanidine and phenamidine had only a slight inhibitory effect on mould development. *A. versicolor* and *A. sydowi* showed very similar reactions to the drug concentrations, which is in keeping with their relationship on morphological grounds. In general, *Penicillium* spp. were not affected by small concentrations (1/2000 or less) of the drugs and were only partially inhibited by higher concentrations (up to 1/125) of sulphonamide E.O.S. Propamidine was more effective against certain *Penicillium* spp. though not all were susceptible. Some of the moulds were slightly stimulated by small concentrations of the drugs. No difference in the fungistatic effect of sulphonamide E.O.S. was apparent at different *p*H levels between 4 and 8.

Different strains of the same species sometimes showed different degrees of resistance or susceptibility to the drug concentrations. This was particularly noted for strains of *Aspergillus ruber*, two of which showed marked resistance to propamidine when incorporated in beer-wort agar while four other strains of the same species were susceptible to the drug when incorporated in this medium. The two resistant strains, however, were susceptible to propamidine when incorporated in the synthetic Czapek-Dox medium.

4 p.p.m. of *p*-aminobenzoic acid was found to annul the fungistatic action of sulphonamide E.O.S. at concentrations of 250 p.p.m. The addition of *p*-aminobenzoic acid to a synthetic medium incorporating propamidine did not annul the inhibitory action of this drug.

Introduction

Drugs of the sulphonamide group have been used extensively in therapeutic practice for the control of bacterial diseases. While numerous publications have given accounts of the effect of these drugs on bacteria, and hypotheses have been put forward as to their mode of action, there has been little published on their effect on moulds.

Dimond (1941), studying a dermatophyte, *Trichophyton purpureum*, showed that 1/1000 of sulphanilamide caused complete inhibition of mat growth. This inhibition was entirely nullified by *p*-aminobenzoic acid in concentrations as low as 1/500,000. Tatum & Beadle (1942) also found that the inhibitory effect of sulphanilamide could be annulled by *p*-aminobenzoic acid for mutant and normal strains of *Neurospora crassa*. Brian (1944) stated that sulphanilamide had a delaying action on the germination and growth of *Penicillium digitatum* Sacc. and on two other mould species: the delaying effect was eliminated by small concentrations of *p*-aminobenzoic acid. These findings are all similar to those of Woods (1940) for bacteria but are confined in their application to only a few moulds.

In view of this limited information regarding the effect of sulphanilamide and, more especially, related drugs on moulds, it was decided to test the reaction of a variety of moulds to some of these drugs. These had all been isolated from feeding-stuffs stored at fixed humidities (Snow *et al.* 1944) and were mainly *Aspergillus* spp. and *Penicillium* spp. It was considered that an investigation of the effect of drugs on these two mould genera might lead to their use for the control of micro-organisms in stored products, since they include the species which most commonly cause damage to stored feeding-stuffs. Subsequent experiments to test the value of the drugs for this purpose are described in a separate paper.

Experimental

Materials and methods

(*a*) *Species of moulds.* During the course of these experiments some fifty distinct mould isolates were used, although not all were employed in any one experiment. They belonged to the following species:

Aspergillus repens (Corda) Sacc.
A. chevalieri Mang.
A. ruber (Spieckermann & Bremer)
A. amstelodami Mang.
A. echinulatus Delacr.

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A. versicolor (Vuill.) Tiraboschi
A. sydowi (Bain. & Sartory)
A. niger Van Tiegh.
Penicillium cyclopium Westling
P. spinulosum Thom
P. luteum series
Two species of *Penicillium* not identified
Paecilomyces varioti Bain.
Alternaria tenuis Nees
Sporotrichum sp.
Rhizopus nigricans Ehrenb.
Cladosporium herbarum Link

The moulds had all been recently isolated from a variety of feeding-stuffs which had been stored under laboratory conditions of controlled humidities for periods of up to 4 years. All were readily identified as typical of these species although sometimes there was considerable individual variation between the isolates of any one species.

(*b*) *Culture media.* Two types of basic media were used: (i) beer-wort agar, which is a rich organic medium containing complex vegetable proteins, and (ii) Czapek-Dox medium, an inorganic medium except for the carbohydrate sucrose. The compositions of the media were as follows:

Beer-wort agar (*p*H 3·5)

(1) Beer-wort	500 ml.	Steamed for 3 days
Water	500 ml.	
Agar	20·0 g.	
(2) Citric acid	12·8 g.	Autoclaved at 10 lb. pressure for 15 min. and then added to (1) above
Potassium citrate	7·2 g.	
Water	50·0 ml.	

Czapek-Dox agar (*p*H 5·5)

Sodium nitrate	2·0 g.
Potassium chloride	0·5 g.
Magnesium sulphate ($7H_2O$)	0·5 g.
Ferrous sulphate ($7H_2O$)	0·01 g.
Potassium dihydrogen phosphate	1·0 g.
Water	1000 c.c.
When dissolved add:	
Sucrose	20 g.
Agar	20 g.

Sterilize by autoclaving at 10 lb. pressure for 15 min.

(*c*) *Drugs used in trials and method of incorporation in media.* The following drugs were tested for their fungistatic properties:

(1) Sulphanilamide — *p*-aminobenzenesulphonamide, $NH_2.C_6H_4.SO_2NH_2$.
(2) Sulphonamide E.O.S.* — Sodium-*p*-aminobenzenesulphonamidoethane-*a*-sulphonate, $NH_2SO_2.C_6H_4.NH.CH.(CH_3).SO_3Na$.
(3) Sulphapyridine — 2:(*p*-aminobenzenesulphonamido)-pyridine, $NH_2.C_6H_4.SO_2NH.C_5H_4N$.
(4) Sulphamezathine — Sulphadimethylpyrimidine, 2-(4′-aminobenzenesulphonamido) 4:6-dimethylpyrimidine.
(5) Sulphaguanidine — *p*-aminobenzenesulphonyl-guanidine.
(6) Propamidine — 4:4′-diamidinodiphenoxypropane di-(β-hydroxyethanesulphonate).
(7) Phenamidine — 4:4′-diamidinodiphenylether di-(β-hydroxyethanesulphonate).

* When dry this highly soluble compound contains 53% of sulphanilamide, which is set free in solution.

The drugs were introduced into the different media in two ways. For drug concentrations of between 1/64,000 and 1/2000 standard amounts of a 0·1% solution of the appropriate drug replaced equivalent volumes of water used in making up the media. For concentrations greater than this amount (i.e. up to 1/125) weighed quantities of the drug were introduced into each flask of medium before sterilization.

(*d*) *Inoculation of plates and colony measurements.* Triplicate plates of each type of medium containing the various drug concentrations were inoculated with each of the moulds used in the different trials.

A spore suspension of each of the moulds was made from an agar-slope culture by shaking with sterile water. A loopful of this suspension was introduced into the centre of each plate. All plates were incubated at 25° C., and observations on the rates of growth of different moulds growing at the various drug concentrations were made by measuring the colony diameters after appropriate intervals of from 3 to 31 days. The diameters were measured in two directions at right angles to each other, and an average value for each plate recorded. Very close agreement was shown in the rate of growth of the triplicate colonies, which seldom showed a variation of more than 1 mm. in diameter. Typical triplicate measurements are shown in Table 1.

TABLE 1. *Triplicate measurements of colony diameters* (*mm.*) *of* Aspergillus sydowi *after* 5 *days*

Drug concentration		Measurements
Control	0	18, 18, 18
Sulphanilamide	1/16,000	17, 17, 17
	1/ 8,000	13, 13, 13
	1/ 4,000	10, 10, 10
	1/ 2,000	5, 5, 7
Propamidine	1/16,000	4, 3, —
	1/ 8,000	— — —
	1/ 4,000	— — —
	1/ 2,000	— — —
Sulphamezathine	1/16,000	15, 16, 16
	1/ 8,000	16, 16, 16
	1/ 4,000	15, 16, 16
	1/ 2,000	14, 14, 12

— indicates no growth.

Effect of sulphanilamide, propamidine and phenamidine on the growth of three Aspergillus *spp. and two* Penicillium *spp.*

As a preliminary trial measurements of the growth of the following five moulds were made on beer-wort agar in which sulphanilamide, propamidine and phenamidine had been incorporated: *Aspergillus repens*, *A. chevalieri*, *A. versicolor*, *Penicillium cyclopium*, *P. spinulosum*.

Each of the drugs was tested at a concentration of 1/16,000, 1/8000, 1/4000 and 1/2000 (i.e. at 63, 125, 250 and 500 p.p.m.). In addition, control plates of the same batch of beer-wort agar in which no drug had been incorporated were inoculated.

Colony measurements were recorded after 4, 6 and 8 days' incubation at 25°C., and the results at 8 days are recorded in Text-figs. 1–3 and in Pls. 6 and 7 (fig. 1).

Phenamidine which is only weakly bacteriostatic, being rather a trypanicidal agent (Lourie & Yorke, 1939; Fulton, 1944), was far less fungistatic than either of the other two drugs. Thus Text-fig. 1 shows that except for some effect on *Penicillium spinulosum* the drug was practically non-fungistatic even at a strength of 1/2000 (500 p.p.m.).

Sulphanilamide was more effective (Text-fig. 2), since it reduced the rate of growth of *Aspergillus repens* and, if present at concentrations greater than 1/4000, completely inhibited *A. chevalieri*. It had no fungistatic effect on either of the *Penicillium* spp. tested, nor on *Aspergillus versicolor*. Indeed, the rate of growth and the production of fructifications of *A. versicolor* were, to a slight extent, stimulated by the presence of small concentrations of the drug.

Penicillium cyclopium was also resistant to the action of propamidine, but this drug was markedly more fungistatic to the other species. It is interesting to note (Text-figs. 2, 3) that, while *Aspergillus versicolor* and *Penicillium spinulosum* were resistant to sulphanilamide, they were even more susceptible to propamidine than were either *Aspergillus repens* or *A. chevalieri*.

Effect of sulphanilamide, sulphonamide E.O.S., *sulphapyridine, sulphamezathine, sulphaguanidine and propamidine on ten isolates of* Aspergillus *spp. and five isolates of* Penicillium *spp.*

Following these preliminary observations a more extensive experiment with six drugs was made, using fifteen moulds as follows:

Aspergillus glaucus *group*

Two strains of *A. repens*
Two strains of *A. ruber*
Two strains of *A. chevalieri*
One strain of *A. amstelodami*
One strain of *A. echinulatus*

Other Aspergilli

A. versicolor
A. sydowi

Penicillia

Paecilomyces varioti
Penicillium cyclopium
P. spinulosum

Two species of *Penicillium* not identified.

Sulphanilamide, sulphonamide E.O.S., sulphapyridine, sulphamezathine, sulphaguanidine and propamidine, were incorporated in a beer-wort agar medium at concentrations of 0 (control), 1/16,000, 1/8000, 1/4000 and 1/2000 for each drug except for sulphonamide E.O.S. which, in addition to these concentrations, was also introduced at a concentration of 1/1000.

The diameters of the mould colonies were measured at 5, 8, 11 or 22 days, according to the rates of growth of the different moulds.

Of the six drugs used, only sulphanilamide, sulphonamide E.O.S. and propamidine had any marked fungistatic effect on any of the moulds tested (see Table 2). The results with *Aspergillus repens*, which were typical for all the *Aspergillus* spp., are recorded in Text-fig. 4, which shows that sulphamezathine, sulphapyridine and sulphaguanidine were only able to effect a very small reduction in the colony size after 11 days' incubation, while sulphonamide E.O.S., sulphanilamide and propamidine had a marked inhibitory effect.

As found in the first trial, the moulds were specific in their reaction to the different drugs. In general, the *Aspergillus* spp. were more affected by the presence of the drugs than the *Penicillium* spp. *P. cyclopium* was unaffected by all concentrations of the drugs except for 1/1000 sulphonamide E.O.S. which reduced the colony size by approximately 50% after 5 days (Pl. 7, fig. 2). The strain of *P. spinulosum* used in this second trial was partially inhibited by concentrations of sulphanilamide and sulphonamide E.O.S. of 1/4000 and above (Text-fig. 5), and thus differed from the strain of the same mould used in the first experiment which was resistant to sulphanilamide. Of the two remaining *Penicillium* spp. which were not identified, the first grew equally well in the presence of any of the drug concentrations, while the second was only partially inhibited by concentrations of 1/4000 and above of sulphanilamide and sulphonamide E.O.S. *Paecilomyces varioti* was unaffected by all the drugs except propamidine at concentrations of 1/4000 and above and sulphonamide E.O.S. at 1/1000. These drug concentrations effected a reduction in colony size of 50 and 25% respectively, compared with the control colony after 5 days' incubation.

As shown in Text-fig. 5 sulphanilamide exhibited a marked inhibitory effect on all members of the *Aspergillus glaucus* group. Sulphonamide E.O.S. had

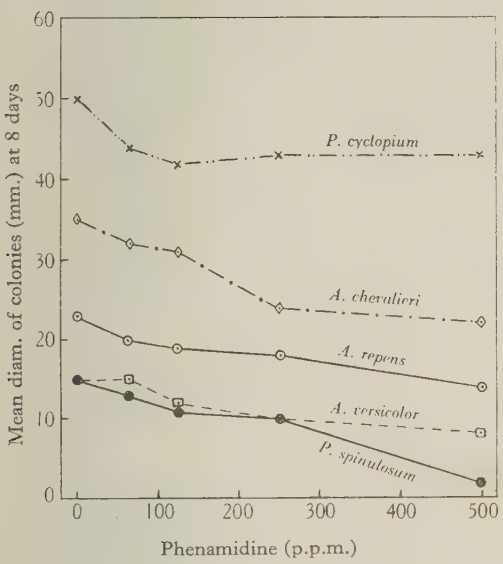

Fig. 1. Effect of phenamidine on 5 mould species.

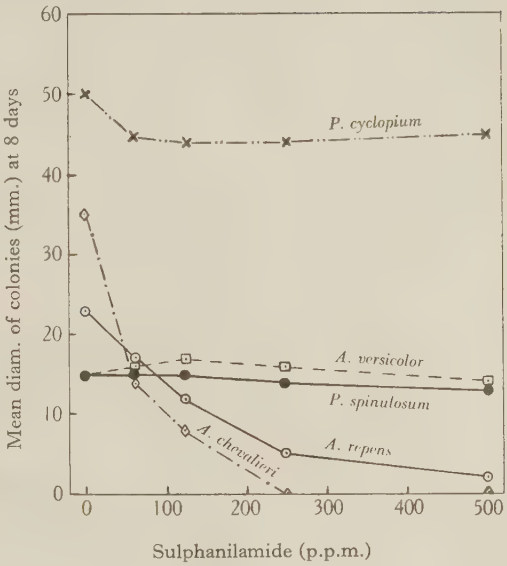

Fig. 2. Effect of sulphanilamide on 5 mould species.

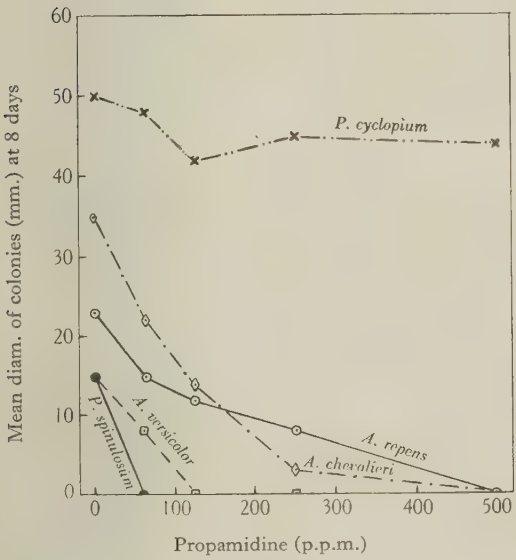

Fig. 3. Effect of propamidine on 5 mould species.

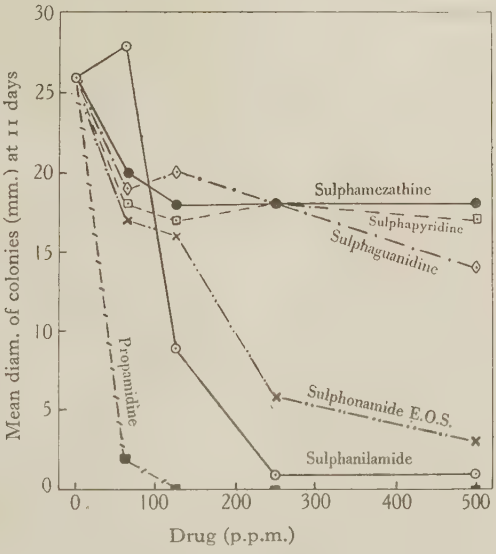

Fig. 4. Effect of 6 drugs on *Aspergillus repens*.

TABLE 2. *Colony diameters (mm.) of* Aspergillus* *spp. and* Penicillium† *spp. at drug concentrations of* 1/8000

	Control	Sulphanil-amide	Sulphon-amide E.O.S.	Prop-amidine	Sulpha-pyridine	Sulpha-mezathine	Sulpha-guanidine
Aspergillus repens (strain A)	26	9	16	0	17	18	20
A. repens (strain B)	13	5	6	0	5	5	1
A. ruber (strain A)	5	2	0	0	3	5	4
A. ruber (strain B)	10	3	3	5	10	8	6
A. chevalieri (strain A)	29	0	11	1	21	22	21
A. chevalieri (strain B)	16	0	10	0	13	13	14
A. amstelodami	26	3	8	0	18	22	25
A. echinulatus	13	0	6	0	5	6	2
A. sydowi	37	26	25	0	30	27	28
A. versicolor	22	19	19	0	18	19	17
Paecilomyces varioti	75	65	70	60	70	70	70
Penicillium cyclopium	18	21	22	23	21	21	20
P. spinulosum	23	23	23	23	23	23	23
Penicillium sp. (not identified)	24	25	24	24	24	24	24
Penicillium sp. (not identified)	18	18	18	18	18	18	18

* 11-day colonies. † 5-day colonies.

a similar action on these moulds. Thus, the presence of 1/4000 of sulphanilamide completely inhibited the development of the strains of *A. repens* and *A. ruber*, while other members of the *A. glaucus* group were even more susceptible, being inhibited by concentrations of 1/8000 and above. On the other hand, the two *Aspergillus* spp. tested which did not belong to this group, *A. versicolor* and *A. sydowi*, showed a marked resistance to sulphanilamide (Text-fig. 5).

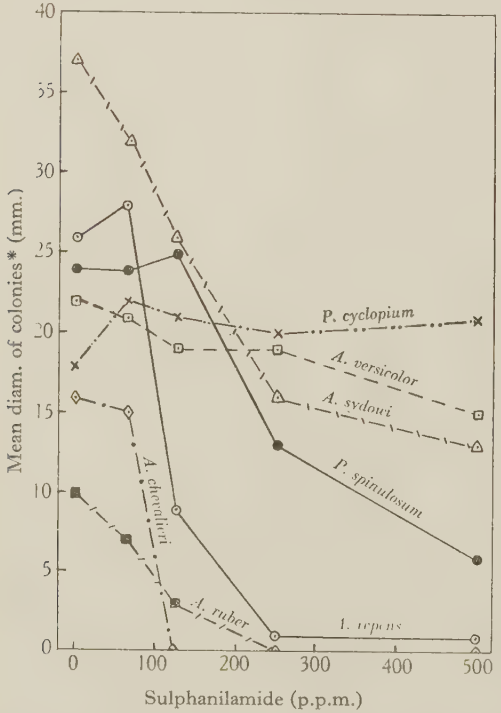

Fig. 5. Effect of sulphanilamide on mould species.
* *Aspergillus* spp. at 11 days. *Penicillium* spp. at 5 days.

Propamidine had the most marked fungistatic effect on the species of *Aspergillus* used. Thus, concentrations of 1/8000 and above of this drug completely inhibited the development of all the *Aspergillus* spp. tested except for one strain of *A. ruber* which appeared to be resistant to propamidine. Some *Aspergillus* spp. were completely inhibited by the presence of only 1/16,000 of the drug.

Effect of higher concentrations of sulphonamide E.O.S. on Penicillium *spp. and certain other moulds*

The experiments which have already been described showed that sulphonamide E.O.S. at small concentrations had but little fungistatic effect on the species of *Penicillium* tested. It was decided, however, to test the reaction of a number of *Penicillium* spp. and other moulds to greater amounts of this drug, and it was therefore incorporated in a beer-wort agar medium at concentrations of 0 (control), 1/8000, 1/4000, 1/2000, 1/1000, 1/500, 1/250 and 1/125. The plates were then inoculated with spore suspensions of *P. cyclopium*, *P. spinulosum*, *P. luteum*, *Penicillium* sp. (not identified), *Paecilomyces varioti*, *Alternaria tenuis*, *Sporotrichum* sp. and *Rhizopus nigricans*. Measurements of the colony diameters were made after 3, 6, 11 and 17 days and figures for the 11-day colonies are recorded in Text-fig. 6. These confirmed the finding of the previous experiments that *Penicillium* spp. were but little affected by concentrations of sulphonamide E.O.S. of 1/2000 and below. Greater concentrations of this drug (up to 1/250), although markedly reducing the growth rate of the *Penicillium* spp., were not able to inhibit completely any of these isolates within the period of the experiment (17 days).

Of the other moulds examined, *Paecilomyces varioti* and *Alternaria tenuis* behaved similarly, but *Sporotrichum* sp. and *Rhizopus nigricans* showed a somewhat different reaction to the various drug concentrations. These moulds were only slightly inhibited at concentrations of less than 1/1000 and 1/2000 respectively, but were completely inhibited by greater concentrations (see Text-fig. 6).

Dox medium whose *p*H was adjusted with *N* HCl or NaOH to four levels—4, 5·5, 7 and 8. The drug concentrations used were 0, 1/8000, 1/4000, 1/2000, 1/1000, 1/500, 1/250 and 1/125. Ten isolates of different mould species were used as follows: *Aspergillus repens*, *A. chevalieri*, *A. sydowi*, *A. versicolor*, *A. niger*, *Penicillium cyclopium*, *P. spinulosum*, *Paecilomyces varioti*, *Cladosporium herbarum* and *Rhizopus nigricans*.

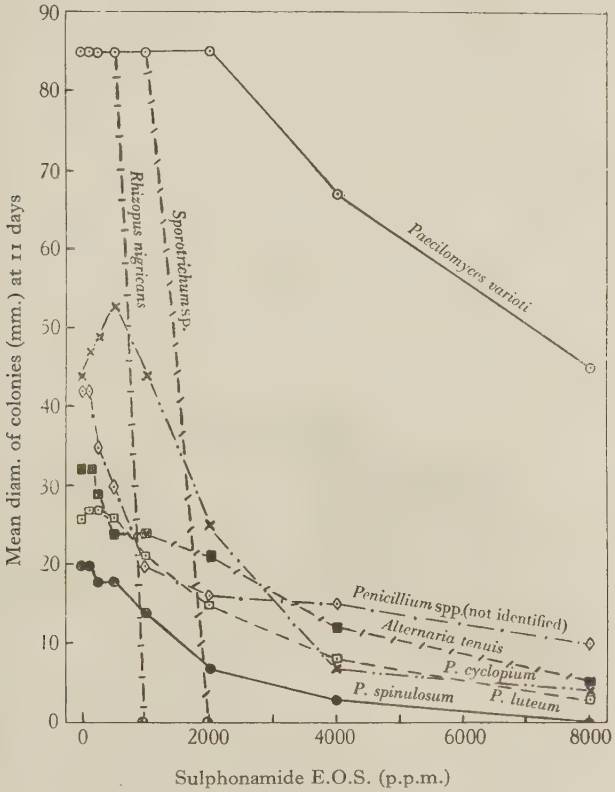

Fig. 6. Effect of higher concentrations of sulphonamide E.O.S. on *Penicillium* spp. and certain other moulds

Fungistatic effect of sulphonamide E.O.S. at various pH levels

Suggestions have been made (Medical Research Council 1943) that, in solution, the activity of the sulphonamides depends upon the anion rather than on the whole molecule. It is stated that the bacteriostatic effect of the drugs tends to be greater in alkaline solutions, since more ions are available under these conditions. Whether this is true for such a highly soluble compound as sulphonamide E.O.S. is not known. However, to test the effect of this drug at various *p*H levels it was incorporated in a Czapek-

Measurements of the colony diameters were made after 7 and 14 days. Although the different *p*H levels resulted in different rates of growth of the moulds on the agar media (the acid *p*H levels allowing more rapid and denser growth), there was no significant difference in the fungistatic effect of E.O.S. at the different levels, it being equally effective whatever the *p*H. Thus the average diameters of the 7-day colonies of *Paecilomyces varioti* measured 62, 58, 38 and 30 mm. for the control plates at *p*H 4, 5·5, 7 and 8 respectively. Text-fig. 7 shows the mean diameters of 7-day colonies of this mould expressed as a per-

centage of the control colony. This method of presentation enables the effect of the drug at the various *p*H levels to be assessed apart from the direct effect of the *p*H. These results are typical of those obtained with all the ten moulds used.

The use of a synthetic medium in this experiment provided an opportunity for comparison of the results of the fungistatic effect of sulphonamide E.O.S. when incorporated in Czapek-Dox medium with those obtained when a beer-wort agar medium was used.

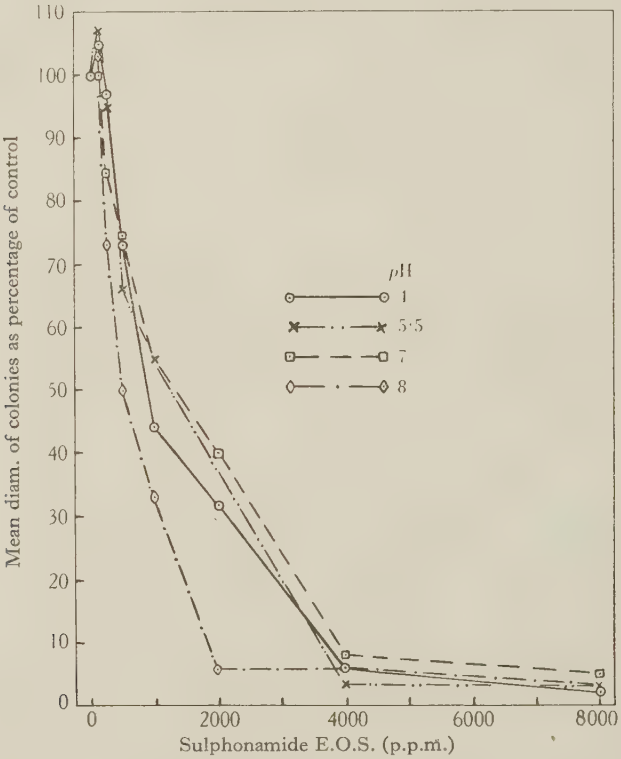

Fig. 7. Effect of sulphonamide E.O.S. at various *p*H levels on the growth of *Paecilomyces varioti*.

It was found that the different reactions of the moulds used in both experiments (i.e. *Aspergillus repens*, *A. chevalieri*, *Penicillium cyclopium*, *P. spinulosum*, *Paecilomyces varioti* and *Rhizopus nigricans*) to the various concentrations of sulphonamide E.O.S. were not affected by using a synthetic medium instead of a beer-wort agar medium.

Of the two species used for the first time in these trials *Aspergillus niger* was partially inhibited by concentrations of 1/4000 sulphonamide E.O.S. and completely inhibited by concentrations of 1/500 of the drug. *Cladosporium herbarum* showed a similar reaction to sulphonamide E.O.S. as did the *Penicillium* spp. used (i.e. it was only partially inhibited by the higher drug concentrations).

Strains of Aspergillus ruber *resistant to propamidine*

It has already been shown that the different mould species used in these trials were mainly specific in their reaction to the various drug concentrations. It has also been pointed out that different strains of the same species (e.g. strains of *Aspergillus ruber* and *Penicillium spinulosum*) showed differences in their reaction to the same drug concentrations.

To investigate the extent of resistance or susceptibility to a single drug (propamidine) within a single mould species, six different strains of *Aspergillus ruber* were selected for experiment. Although the six strains showed marked differences in rate of growth, colony habit, predominance of either green conidial fructifications or yellow-red cleistothecia, etc., they were clearly grouped together under the single species because each strain showed the small ascospores with a broad shallow furrow bounded by low rounded equatorial ridges and no crests which

characterize this species. All strains produced a red pigment in the medium. The strains of *A. ruber* were grown on two types of medium, beer-wort agar and Czapek-Dox agar, each with propamidine incorporated at concentrations of 0 (control), 1/64,000, 1/32,000, 1/16,000, 1/8000, 1/4000 and 1/2000. Measurements of the mould colonies were made after 7, 14 and 21 days and figures for the 21-day colonies are recorded in Table 3. It is clear that while strains A, B, C and F were susceptible to even the smallest concentration of propamidine (1/64,000), strains D and E showed a marked resistance to concentrations

this action, since it has already been shown by Dimond (1941), Tatum & Beadle (1942) and Brian (1944) that this substance will prevent the inhibitory action of sulphanilamide on moulds. To ascertain if this could explain the observed effects of propamidine on different strains of *A. ruber*, *p*-aminobenzoic acid was added to inhibitory media.

p-Aminobenzoic acid as an anti-sulphanilamide agent

Propamidine and sulphonamide E.O.S. were each incorporated into Czapek-Dox medium in concentrations of 0 (control), 1/64,000, 1/32,000, 1/16,000

TABLE 3. *Effect of propamidine on the diameters of 21-day colonies (mm.) of six strains of* Aspergillus ruber

	Strains of *A. ruber*											
	A		B		C		D		E		F	
Conc. of propamidine	Bw	C-D	Bw	C-D	Bw	C-D	Bw	C-D	Bw	C-D	Bw	C-D
0 (control)	15	3	9	1	16	7	25	15	19	21	24	8
1/64,000	—	—	—	—	—	—	27	10	20	—	—	—
1/32,000	—	—	—	—	—	—	26	—	15	—	—	—
1/16,000	—	—	—	—	—	—	24	—	17	—	—	—
1/8000	—	—	—	—	—	—	23	—	18	—	—	—
1/4000	—	—	—	—	—	—	23	—	2	—	—	—
1/2000	—	—	—	—	—	—	—	—	—	—	—	—

Bw = beer-wort medium; C-D = Czapek-Dox medium; — = no growth.

TABLE 4. *The effect of the addition of* p*-aminobenzoic acid to inhibitory media on the diameters of 7-day colonies (mm.) of* A. ruber

	Conc. of drug (p.p.m.)								
Conc. of *p*-aminobenzoic acid (p.p.m.)	0 (control)	15		31		63		250	
		S	P	S	P	S	P	S	P
		A. ruber, strain D							
0	5	1	3	0	0	0	0	0	0
4	5	4	1	4	0	4	0	4	0
15	5	5	1	5	1	4	0	4	0
63	5	5	0	5	1	5	0	5	0
		A. ruber, strain E							
0	6	0	1	0	0	0	0	0	0
4	5	6	1	2	0	6	0	5	0
15	6	5	1	5	0	3	0	3	0
63	6	5	0	5	0	3	0	2	0

S = sulphonamide E.O.S.; P = propamidine.

of the drug as high as 1/4000 when incorporated in beer-wort agar (see Pl. 7, fig. 3). These same strains of *A. ruber* (D and E) were, however, susceptible to propamidine incorporated in the Czapek-Dox medium (Table 3 and Pl. 7, fig. 4), even though they grew quite vigorously (and strain E grew even more vigorously) on the control plate of this synthetic medium compared with beer-wort medium.

It appears, therefore, that strains of *A. ruber* vary in their metabolic requirements, and that there is present in beer-wort agar some substance that is able to nullify the inhibitory action of propamidine on certain strains of this mould and not on others. It is possible that *p*-aminobenzoic acid is concerned in

and 1/4000. *p*-Aminobenzoic acid was added at four concentrations, 0, 1/256,000, 1/64,000 and 1/16,000, to each of the drug types. These concentrations are equivalent to 0, 15, 31, 63 and 250 p.p.m. of the drugs and to 0, 4, 15 and 63 p.p.m. of *p*-aminobenzoic acid. Triplicate plates of each type of medium containing the range of drug and *p*-aminobenzoic acid concentrations were inoculated with a spore suspension of two strains of *A. ruber* (D and E) used in the previously described experiment.

The colony diameters were measured after 7, 14 and 21 days, and Table 4 records the measurements made after 7 days. The marked inhibitory effect of sulphonamide E.O.S. on the growth of both strains

of *A. ruber* was completely annulled by the presence of *p*-aminobenzoic acid even at the smallest concentration used. This concentration (4 p.p.m.) enabled colonies growing on media containing as much as 250 p.p.m. of sulphonamide E.O.S. to attain the size of the control colony.

Propamidine incorporated in the Czapek-Dox medium effected marked inhibition of both strains of *A. ruber* at all the drug concentrations used. *p*-Aminobenzoic acid did not annul this inhibition (see Table 4), which agrees with the results of Thrower & Valentine (1943) for bacteria.

Discussion

Specificity of reaction of different moulds to the drugs

The experiments described have enabled the reactions of a variety of moulds to sulphanilamide and other similar drugs to be assessed. One of the most noticeable features was the specificity of reaction to the different drugs that each of the moulds showed. Not only did different groups, genera and species of moulds differ in reaction to the various drug concentrations, but also different strains of the same species showed various degrees of resistance or susceptibility. In view of this observation it is difficult to arrive at any definite conclusions on the reaction of particular moulds to the drugs used. In general, however, it was found that *Aspergillus* spp. were inhibited to a greater extent than *Penicillium* spp. While very small concentrations (1/8000) of some of the drugs, notably sulphanilamide and propamidine, completely inhibited the growth of members of the *Aspergillus glaucus* group, it was found that these concentrations had little inhibitory effect on the development of *Penicillium* spp., *Paecilomyces varioti*, *Alternaria tenuis*, *Rhizopus nigricans* and *Cladosporium herbarum*. Even high concentrations of sulphonamide E.O.S. of up to 1/125 only partially inhibited these moulds.

Resistance to the drugs was not a function of speed of growth rate of the different strains or species tested. Thus, fast-growing strains of *Aspergillus chevalieri* were more susceptible to sulphonamide E.O.S. than much slower growing isolates of *Penicillium luteum* and other species that were resistant to this drug. Resistance and susceptibility to the drugs must therefore be attributed to other physiological characters which are typical of the different metabolic processes of the various moulds. Certain group relationships were noted for the reaction of some of the moulds tested. Thus, the species included in the *Aspergillus glaucus* group showed very similar reaction to the different drug concentrations: most were inhibited by small concentrations (1/8000) of both propamidine and sulphanilamide. Other *Aspergillus* spp. which were used in these experiments (*A. sydowi* and *A. versicolor*), although inhibited by propamidine at concentrations as small as 1/16,000, were both markedly resistant to sulphanilamide at concentrations less than 1/2000. These two species were also only partially inhibited by concentrations of 1/1000 and below of sulphonamide E.O.S. but were inhibited by greater concentrations (1/500) of this drug. That these two species show similar physiological reactions to the drugs is of interest because of their agreed relationship on morphological grounds (i.e. the arrangement of sterigmata in two series) and their inclusion by Thom & Church (1926) in the same morphological group. *Aspergillus niger*, *Rhizopus nigricans* and *Sporotrichum* sp. were also found to be resistant to concentrations of 1/1000 and below of sulphonamide E.O.S., though they were completely inhibited by higher concentrations.

Most of the *Penicillium* spp. tested were highly resistant to all concentrations of the drugs. Small concentrations had little or no effect on the growth rate of most of the species tested, and where the drugs were present in larger concentrations (i.e. up to 1/125 of sulphonamide E.O.S.) complete inhibition of these species was generally not obtained, although a gradual reduction in the rate of growth was observed in relation to the amount of drug present in the medium. A similar reaction to the drugs was shown by *Paecilomyces varioti*, which species is included by Thom (1930) in the Penicillia, and also by *Alternaria tenuis* and *Cladosporium herbarum*.

Stimulatory effects of small concentrations of the drugs

It may be noted that for some of the moulds small concentrations of sulphanilamide or sulphonamide E.O.S. had a slight stimulatory effect on the rate of growth compared with the control colonies. For *Penicillium cyclopium* this stimulation could be measured by small increases in the colony diameters which are recorded graphically in Text-figs. 5 and 6. For some other species, particularly *Aspergillus versicolor*, *A. niger* and *Penicillium spinulosum*, while no increase in the colony diameters was measurable, small concentrations of sulphanilamide or sulphonamide E.O.S. encouraged more rapid development of a denser mould colony with a more extensive production of asexual fructifications (conidia) compared with the control colonies. Such stimulatory effects are similar to those resulting from the presence of small concentrations of heavy metals and of other substances which are toxic if present in larger amounts, as described by Brenchley (1914) and other authors.

Resistant and susceptible strains of a single-mould species

The experiments have shown that within a single-mould species many degrees of resistance or susceptibility to sulphanilamide and the other drugs may exist. Particular study was made of strains of *Aspergillus ruber*, some of which showed high degrees of resistance to propamidine, while most of the strains were susceptible to small concentrations of this drug.

It is possible that previously susceptible strains of this and other mould species may acquire resistance to sulphonamide drugs including propamidine, as has been shown for several types of bacteria. The experiment which demonstrated the existence of resistant and susceptible strains of *A. ruber* showed, moreover, that the type of culture medium used for testing the resistance of strains of moulds to the drugs influences the results obtained. Thus while two strains of *A. ruber* were highly resistant to propamidine incorporated in a beer-wort agar medium they were completely inhibited when grown on a synthetic medium containing the same drug concentrations. This may be accounted for in two ways. First, the fungistatic action of this drug may be controlled to some extent by the type of the medium in which it is incorporated, and particularly by the nature and amount of the nutrients present. Secondly, it is possible that the beer-wort medium contained some agent, not present in the synthetic medium, which specifically antagonized the fungistatic action of propamidine on the two resistant strains of *A. ruber* but which was not functional for the four susceptible strains.

Anti-sulphanilamide effect of p-*aminobenzoic acid*

Woods (1940) showed that yeast extracts antagonized the inhibitory effect of sulphanilamide in bacterial cultures and further showed that *p*-aminobenzoic acid had a similar action which he regarded as identical to the yeast factor. Other authors have shown that other substances may also have antagonistic effects on the anti-bacterial action of the sulphonamides (see Henry, 1943). Experiments carried out in this present series have shown that very small amounts of *p*-aminobenzoic acid are able to annul the fungistatic action of sulphonamide E.O.S. Thus, the presence of only 4 p.p.m. of *p*-aminobenzoic acid completely antagonized the action of 250 p.p.m. of sulphonamide E.O.S. *p*-Aminobenzoic acid can, therefore, act as an anti-sulphanilamide factor for moulds as well as bacteria, and, following upon the suggestion of Woods (1940), may be an essential metabolite for mould growth. This substance was not, however, able to counteract the fungistatic effect of propamidine, the active radicle of which is 4:4′-diamidinodiphenoxypropane, and it is, therefore, probable that this drug follows a different mechanism from that of sulphanilamide, or affects a different substrate.

General applications

These investigations on the reaction of moulds to sulphanilamide and other therapeutic drugs are in no way complete. Further work is necessary before any definite conclusion can be drawn on the mechanism of the inhibitory action of the drugs on moulds and their relationship to the metabolism of such organisms. But an immediate practical application is whether these substances can be used to prevent or delay deterioration of feeding-stuffs and other stored products by moulds. In this connexion accelerated laboratory tests have established that the storage life of samples of ground linseed cake incorporated with 0·2% sulphonamide E.O.S. can be doubled when stored at 85 and 80% R.H. and at 25° C. Where the outsides of the cakes were sprayed with a 2% solution of this drug the length of storage was increased as much as three- to four-fold compared with untreated cakes stored at humidities between 100 and 80%. Similar applications have been made to small cheeses under laboratory conditions, where dipping in a 2% sulphonamide E.O.S. solution before larding has more than doubled the storage life of cheeses stored at humidities between 100 and 80%. Details of such investigations are reported in a separate paper.

The authors wish to express their thanks to Imperial Chemical (Pharmaceuticals) Ltd. and to May and Baker Ltd. for providing the drugs used in these experiments.

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EXPLANATION OF PLATES 6 AND 7

PLATE 6

Inhibitory effect of sulphanilamide and other drugs on mould species growing on beer-wort agar plates (8-day colonies).

Fig. 1. *Aspergillus repens* (Corda) Sacc.

Fig. 2. *Aspergillus chevalieri* Mang.

Fig. 3. *Aspergillus versicolor* (Vuill.) Tiraboschi.

Fig. 4. *Penicillium cyclopium* Westling.

Top row of plates: sulphanilamide. Middle row of plates: propamidine. Bottom row of plates: phenamidine. Arranged from left to right in drug dilutions of 0 (control), 1/16,000, 1/8000, 1/4000 and 1/2000.

PLATE 7

Fig. 1. *Penicillium spinulosum* Thom. For description see Pl. 6.

Fig. 2. Effect of 1/1000 sulphonamide E.O.S. on the growth of *Penicillium cyclopium* Westling (6-day colonies) (×2). Left-hand colony: control grown on normal beer-wort agar. Right-hand colony: colony grown on agar containing 1/1000 sulphonamide E.O.S. (Note reduction in size and buckling of colony.)

Fig. 3. Resistant and susceptible strains of *Aspergillus ruber* (Spieckermann & Bremer) to propamidine incorporated in beer-wort agar. Top row of plates: strain C, susceptible. Bottom row of plates: strain D, resistant. Arranged from left to right in dilutions of propamidine of 0 (control), 1/32,000, 1/8000, 1/4000 and 1/2000.

Fig. 4. Resistance and susceptibility of a single strain (strain E) of *Aspergillus ruber* (Spieckermann & Bremer) to propamidine incorporated in beer-wort and Czapek-Dox medium. Top row of plates: propamidine incorporated in beer-wort medium. Bottom row of plates: propamidine incorporated in Czapek-Dox medium. Arranged from left to right in dilutions of propamidine of 0 (control), 1/64,000, 1/32,000, 1/16,000 and 1/8000.

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Certain aspects of resistance of plum trees to bacterial canker

Part II. On the nature of the bacterial invasion of *Prunus* sp. by *Pseudomonas mors-prunorum* Wormald

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A detailed histological study was made of the necrotic areas induced in the stem of a resistant and a susceptible variety of plum as the result of artificial inoculations of *Ps. mors-prunorum* Wormald. The pathogen was found to penetrate the tissues intercellularly, causing plasmolysis of cell contents, followed by disintegration of cell walls, and gradual invasion of the bacteria. The seasonal progress of infection was followed, the maximum cankers caused by winter inoculations in the susceptible host appearing in spring. Limitation of necrosis occurs in summer, generally accompanied by the production of new tissue composed of xylem elements. Viable bacteria were isolated in autumn from those cankers in which the area of necrosis had extended into the wood cylinder beyond the limiting periderm. The difference in the nature of the injury to the tissues of the resistant trees is quantitative rather than qualitative. The significance of the apparently more efficient host mechanism of periderm production in this variety is considered. Lesions similar in histological aspect were obtained as the result of the injection of cell-free filtrates of cultures of the causal organism.

INTRODUCTION

In a previous paper (Erikson, 1945) the cultural characteristics of *Pseudomonas mors-prunorum* Wormald, the causal organism of bacterial canker of plum in this country, were considered in relation to those of other related phytopathogenic bacteria. The present study deals with the mode of invasion of *Ps. mors-prunorum* within the living tissues of both a susceptible and a resistant variety of host, with factors influencing the spread and longevity of the inoculated organism, and with the host reactions to this invasion.

EXPERIMENTAL MATERIAL

Two varieties of 3-year-old plum trees were used for inoculation experiments: susceptible (Giant Prune) and resistant (Warwickshire Drooper). One strain only (A61) of *Ps. mors-prunorum*, which has already been described (Wormald, 1932; Erikson, 1945), was employed throughout the main body of the work. The cultures of this organism, originally derived from a single colony, before being used for inoculation purposes were repeatedly subcultured in nutrient broth for the enhancement of virulence. A few drops

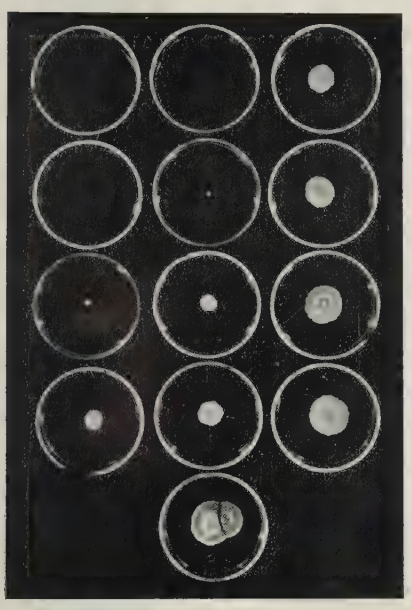

Fig. 2

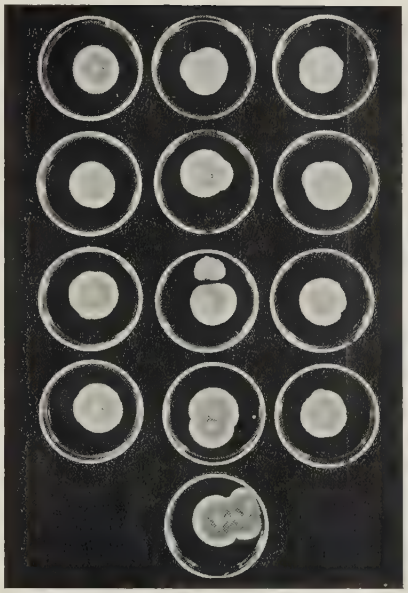

Fig. 4

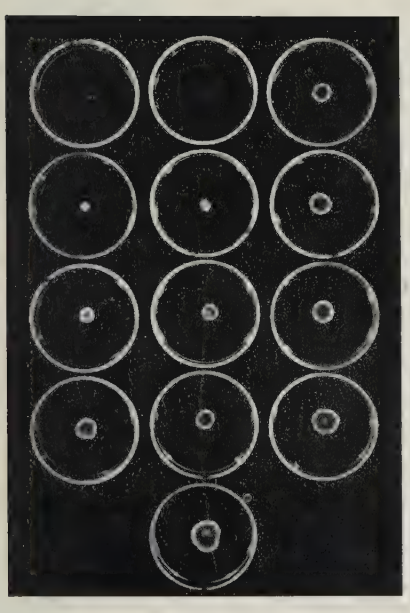

Fig. 1

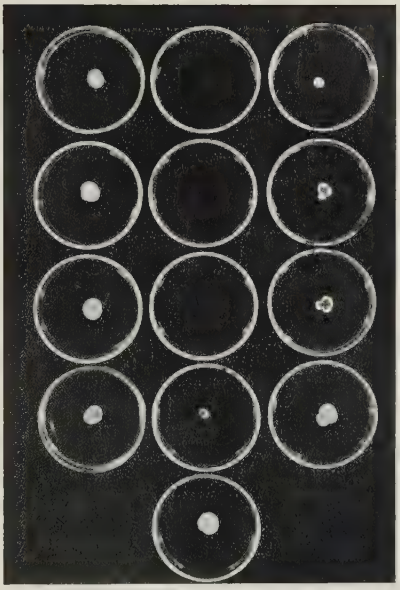

Fig. 3

SNOW AND WATTS—EFFECT OF SULPHANILAMIDE

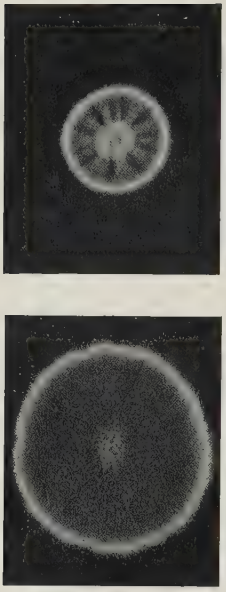

Fig. 2

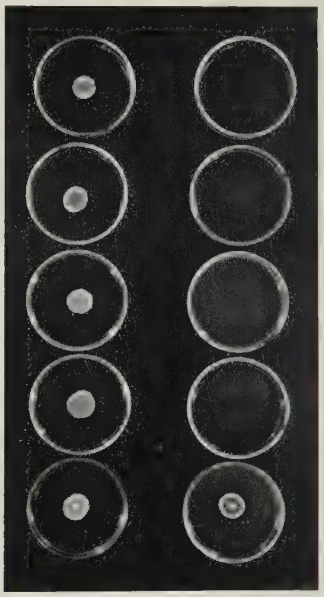

Fig. 4

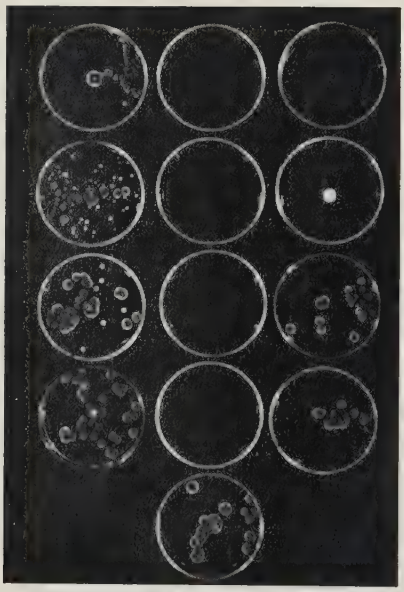

Fig. 1

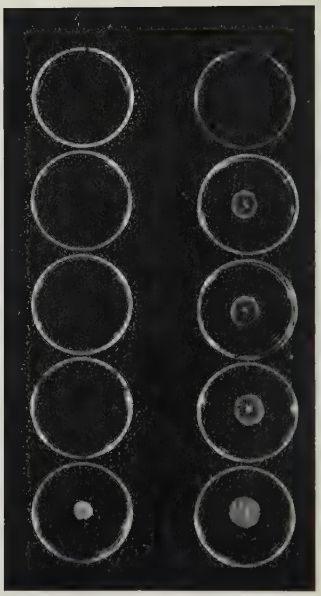

Fig. 3

SNOW AND WATTS—EFFECT OF SULPHANILAMIDE

of the aqueous suspension obtained from a 2-day-old nutrient agar slope were introduced into the stem of the tree according to the method described by Wilson (1933). At frequent intervals the cultures were plated and examined for purity. Commencing in December 1941, the susceptible trees were inoculated at 4–5 places on the stem at 3–4 weekly intervals until June 1942, and the resistant trees at the two peak periods, December and June. Control trees received an incision with a sterile needle.

Histological technique

Freehand sections. Freehand sections of fresh bark taken from the vicinity of the sites of inoculation were made throughout the period. These were fixed in formalin-acetic-alcohol and stained with very dilute aqueous methylene blue (1 : 10,000). The selectiveness of the stain at this dilution (Johansen, 1940) results in lignin being coloured green, cellulose blue, and pectic substances violet. Excellent differentiation between the healthy and diseased portions of the phloem was also secured by this means, and it was consistently noted that in the latter areas only the green of the lignin in the sclerenchyma bundles remained unaffected within the general brown discoloration.

Certain other microchemical tests, using the methods of Johansen, were also made. It was found that the hydrolysis test for cellulose did not give as demonstrably clear results in the necrotic as in the healthy areas, especially when there was considerable tissue disintegration; nor could the presence of methyl pentosans (rhamnose) be detected, although repeatedly discerned in normal tissue. A negative test for arbutin was given with both healthy and diseased tissues. In general, however, it was difficult to detect any colour changes in the macroscopically dark necrotic area, apart from the blackening resulting from the formalin-ferric chloride test which demonstrated abundantly in both susceptible and resistant barks the presence of tannins (Johansen) or other phenolic constituents (Rawlins & Thomas, 1941).

Paraffin sections. After many trials the *n*-butyl alcohol-cedar oil-paraffin technique of Rawlins & Takahashi (1941) was found most satisfactory. Although laborious and time-consuming, this method permits smooth ribbons of 4–10 μ thickness to be cut on a rotary microtome, and the sections show very little distortion. The paraffin blocks, when trimmed so as to expose one cut surface of the embedded object, are then soaked in cold or ice water for 1–3 days for further softening before being cut. Frequent application of an ice-block to the paraffin surface during the process of cutting is often a help.

Various staining schedules were employed, the most satisfactory being safranin counterstained by Harris's haematoxylin, methyl violet counterstained by erythrosin, and safranin counterstained by fast green (Johansen, 1940). Good results were also obtained with the Milovidov (1928) technique for the differentiation of mitochondria and bacteria, which required his special neutral fixative to give a basic fixation image.

Several hundred sections were prepared, from the examination of which the following description is compiled.

Seasonal reaction of the host towards inoculation of the living pathogen

(*a*) *Susceptible host*

Winter (dormancy up to the first signs of cambial activity). Exceptionally cold weather was experienced during the winter of 1941–2, and no macroscopic lesions were apparent at the beginning of March. At this period microscopical examination of the December inoculations revealed that the bacteria had made little progress beyond the needle track. A small portion of the phloem and some of the cambium were destroyed, but in general the xylem was not invaded. The bacteria penetrate between the cells but do not invade them until the cell walls begin to crumple and disintegrate and the cell contents become plasmolysed.

This movement of the bacteria between the cells in a zoogloeal mass in which the component bodies can be discerned as very short rods only with high magnification (× 1000) and with considerable difficulty because of the enveloping matrix, is in conformity with the findings of Robinson & Walkden (1923) and of Hill (1928) in connexion with the invasion of the crown-gall organism (*Bact. tumefaciens*) and also of Nixon (1927) and of Thomas & Ark (1939) for the fire-blight organism (*Bact. amylovorus*).

Material inoculated in February and fixed in early April showed irregular invasion of the xylem by the bacterial mass, resulting in plasmolysis of the cell contents and disintegration of the cell walls of the medullary rays. Early in April there may also be seen the beginnings of a phellogen—meristematic cells with the nuclei well marked—representing the onset of cambial activity in spring and the first signs of host response to invasion. No cork cells have yet been produced by the phellogen, which at this stage is only two cells deep.

Spring (greater involvement of tissues, stimulation of new growth, production of periderm). The host response to a wound made in early spring is greater, and the active nuclear contents of newly developed meristematic tissue can be seen staining very clearly. Examination during this period of bark inoculated in winter reveals a greater area of necrosis which is clearly defined by the growth of new tissue arising in the neighbourhood of the cambium. A true periderm of cork cells only has not yet been formed except to a very small extent, and the cells limiting the lesion re-

semble those of the interior in having the characteristic plasmolysed, deeply staining contents bounded by thickened cell walls. No bacteria can be discerned within these diseased cells, but among the disintegrated tissue within the necrotic area are a few free-lying patches of bacteria. The pathogens are present in considerable numbers in the small V-shaped apex of invasion (regarding the section radially with the necrotic area extending in depth towards the central wood cylinder). Here the cambial cells are destroyed but the xylem is not invaded, and young tissue is dividing actively round this small core of diseased tissue and in the course of separating it from the healthy phloem by means of a periderm.

Towards the end of May, when the cambial activity of the host is still greater, the necrotic area may be further advanced in disintegration, but it is then outlined by a regular periderm of several cells in depth, the meristematic tissue having proliferated to a greater extent around the apex of invasion which, however, can often be seen extending into the wood. Such small foci of invasion in the xylem may then be isolated from the healthy tissue by means of abundant cell division. As a result of the pressure of this growth the original periderm is often ruptured more or less completely, causing the diseased area to be dissociated from the surrounding tissue. In the xylem, also, the method of invasion is *inter*-cellular.

Specimens inoculated and fixed 20 days later in May have shown a complete periderm many cells deep surrounding the necrotic area, with the exception of the apex of invasion leading to the wood. Small patches of diseased tissue outside this corking sheath have in some instances again been marked off by a second periderm of thick-walled empty cells. It is to be noted that the bundles of hard-walled sclerenchyma which are characteristic of the bark of the plum are rarely affected by the invasion of the pathogens, or split apart by the production of a periderm delimiting such invasion.

In fresh material certain discoloured streaks in the upper layers of the cortex are to be seen extending upwards and downwards from the lesion. This is due to the extension of one or two rows of cells with darkly staining, degenerate contents originating from a patch of diseased tissue outside the periderm.

Such a phenomenon is reminiscent of that second type of canker described by Wilson (1939) as often characteristic of *Prunus salicina* varieties 'in which the margins were well defined at the edges of the necrosed area and which also possessed a zone of reddish-brown dormant streaks beyond the necrosed area', in contrast to the type occurring more often in *Prunus domestica* varieties 'in which the dull brown, watery margins gradually merged into healthy tissue, with no reddish-brown dormant streaks'. But in the Californian experience of this worker such 'dormant streaks' (examined, however, during the second season after the establishment of infection, not, as here, during the first) were many cells in depth, surrounded by periderm, and full of viable bacteria. In the present instance no bacteria could be isolated from, nor detected microscopically within, this narrow area, and the degenerate cells have the same aspect as those of similar streaks resulting in this variety of plum from the injection of cell-free culture filtrates of the pathogen.

Summer (limitation of necrosis, production of callus). In June the morbid tissue resulting from winter inoculations is often readily detachable by any slight pressure, which ruptures the surrounding periderm from the new-formed tissue found below. This new-formed tissue is, as was first stated by Wilson, xylem, and consists of thin-walled secondary cambial cells with well-marked, active nuclear contents, medullary ray cells packed with starch grains, and new vessels with bordered pits round a large air space. The pathogens are found abundantly in the central necrotic area adjoining that part of the xylem which has not been entirely overlaid by new tissue. Extensive damage to the xylem results in the collapse of some of the parenchymatous cells; some spiral tracheides are occluded with gum or other opaque, dark-staining masses so that only the spiral thickenings are discernible; and while many medullary ray cells may still be packed with starch grains, others are empty or contain only the products of degeneration. Aderhold & Ruhland (1907), in their pioneer work on bacterial canker of cherry, also noted that the medullary rays were commonly resistant to disintegration, the spaces between them being filled with gum and broken-down cells.

Inoculations made in June and July, the so-called resistant period, give smaller lesions which are as a rule confined to the phloem and quickly overlaid by new growth. A macroscopic difference exists at this period between cankers in susceptible and resistant hosts, both inoculated in December: the former are depressed, water-soaked areas with a more or less clear line of demarcation; the latter are recognizable by irregular protuberances, which are rarely water-soaked, but which may be covered by gum; whereas in both varieties sterile control punctures leave a smooth line.

No leaf-spots, described by Wormald (1937) as important oversummering sources of infection, were encountered, and all inoculations into green shoots and leaves failed. This is probably to be ascribed to the unusually dry weather of this season.

Autumn (decline of cambial activity). None of the cankers still remaining from the preceding winter's inoculations showed any sign of recrudescence of activity at this period. When examined at the end of November, the depression in the susceptible bark was no longer clearly defined, and the protuberance in the resistant stem had as a rule ruptured. Inoculations made throughout September and up to the

third week of October resulted in the production of periderm, but towards the end of October there was a gradual cessation of all cambial activity. No periderm was produced as the result of November inoculations in either susceptible or resistant host.

(*b*) *Resistant host*

The lesion obtained in spring or summer as the result of winter inoculations into the resistant tree is in general very small, confined to the phloem, and very effectively isolated by a many-layered periderm. The new tissue which has been produced to bury the necrotic area is again xylem, but this is more irregularly laid down than is usually the case in the susceptible host. More extensive lesions show the abundant development of periderm round the main and any secondary infection, and also the existence of certain discoloured streaks outside the periderm.

The aspect of the cells in these streaks differs in certain minor details from that described above for the susceptible trees. There is an intercellular penetration of very densely staining material in which bacterial rods can hardly be discerned, but which takes the bacterial counterstain in the same way as indubitable patches of bacteria within the main lesion; and the contents of the cells comprising the streak are not absolutely opaque and plasmolysed in an irregular rounded mass as is common in the susceptible host, but fragmentary and only partially stained. It is possible that such an area represents a further invasion of bacteria which took place shortly after the prolongation of the periderm about the secondary infection, and that the bacteria died off (no successful isolation from resistant trees was obtained at this period, August) before any appreciable damage could be done. This corresponds with Wilson's observation that 'diseased areas that have entered the inactive state with little or no partially invaded tissue at the edge of their uniformly necrosed margins' rarely contain viable bacteria and are usually bordered by callus.

The comparative paucity and, where present, the relative smallness in extent of these discoloured streaks in the phloem, and the general infrequency or absence of browning in the xylem on the part of the resistant host may indicate that the pathogen in the resistant tree fails to produce, at least in comparable quantities, metabolites deleterious to the plant tissues. Alternatively the resistant host may not easily be affected by metabolites harmful to the susceptible one.

The production of gum is much more frequent and copious in this resistant variety than in the susceptible one, and often follows non-parasitic wounding. Plum gum when tested against the pathogen in pure culture was found to be without a toxic effect at a concentration of 5%. It therefore seems probable that if the gum plays any part in resistance, it is only as a mechnical factor due, perhaps, to its physical properties of swelling in water and so blocking the progress of invasive bacteria (cf. the 'gum barriers' of Brooks & Moore (1926) in the silver-leaf disease).

Longevity of inoculated organism in host

No difficulty was experienced in reisolating the organism from either host at any time between December and June. In July it became evident that viable bacteria were much more infrequent (cf. Wormald, 1937), and attempts at isolation from the comparatively small lesions in the resistant tree were thereafter unsuccessful. In the case of the susceptible host, it was found that by carefully separating small strips of diseased phloem and xylem, incubating them separately in broth, and then making dilution plates on a synthetic sucrose-nitrate medium to discourage the large number of saprophytes present at this period, successful isolations could be secured throughout August, September, and, less often, October and November. Such isolations were successful only in the case of cankers in which the xylem was involved, and usually only from the wood. Thus, it seems clear that a few bacteria may survive in the deeper-seated tissues long after the majority have died in the main canker, and it is possible that given favourable conditions these may serve as foci of fresh infections in the autumn. That was not the case in the present instance. Viable bacteria were not isolated in the following spring of 1943, nor was any extension then observed of any of the old-established lesions which had reached their maximum in the summer of 1942.

It is difficult to attribute this dying of the bacteria to any lack of an adequate food supply, in view of the very simple nutritive requirements of *Ps. morsprunorum* described in the previous paper (Erikson, 1945). The organism also is capable of growth between *p*H 5 and 8, a broad biological range. It would seem, therefore, that physical factors or a toxic accumulation of the products of degeneration are more likely to be responsible.

Host reaction towards injection of bacterial metabolites

In an attempt to discover whether substances toxic to the host are elaborated *in vitro* by pure cultures of the pathogen, cell-free filtrates of cultures of varying ages on different synthetic and bark-extract media have been prepared and injected into both varieties of host at different seasons of the year. These experiments will be described elsewhere, and here attention is confined to the histological aspect of the lesions resulting from such injections. Early plasmolysis of the phloem cells was constantly observed. Such 'interference with the water retention in plant cells by materials toxic to the tissues' has been noted by Thomas & Ark (1939) in the case of the fire-blight

disease. On the whole, the damage done to the phloem by the introduction of these cell-free filtrates was less extensive than in the case of the inoculation of the living bacteria, and actual breakdown of the cell walls and complete disintegration of the cells were less frequently encountered. On the other hand, where cambial degeneration and entry into the xylem were effected, a very considerable necrosis resulted. Sections of such extensive brown streaks in the wood gave pictures comparable in all respects with those produced by the invasion of living bacteria.

Some experiments were also made in introducing into the host the bacterial polysaccharide produced by *Ps. mors-prunorum* from sucrose. Alone, the crude polysaccharide caused little more than a local, circumscribed injury in the susceptible bark. When it was injected together with a suspension of living bacteria in early autumn, accelerated cork formation round the lesion was observed, as compared with the slower, regular rate of periderm production occurring in response in the control inoculation of the pathogen only. When, however, a purified specimen of the levan was injected the following spring in the same manner, these results were not confirmed, and the earlier stimulation of periderm production may thus have been due to some impurity or seasonal variation. The lack of toxic effect of the polysaccharide in the host was confirmed with the purified specimen.

Role of periderm in control of canker expansion

The fact that viable bacteria occurring in the susceptible host could be isolated late in the season as a rule only from the deeper-seated tissues which were not corked off, or from the neighbourhood of the cambium where the apex of invasion had advanced beyond the periderm, suggests that periderm production may be a factor in checking canker extension. Indeed, the comparable aspect of the usually small, well-corked lesion in the resistant outer bark with that of those necrosed areas in the susceptible host, which have been effectually isolated in the phloem before any invasion of the xylem has taken place, makes it clear that the spread of the living organism can under certain circumstances be restrained by this host mechanism. On the other hand, no significant difference was found between susceptible and resistant hosts as regards the first appearance of periderm at the end of April or its decline at the end of October, although Wilson, working with a greater variety of trees, found such differences. But it is possible that factors obtain in the resistant host which render it more readily and more consistently able to react to external stimuli in general. The greater frequency of gum production after mechanical wounding in this variety could be adduced as an argument in favour of this thesis. A further point not to be overlooked is the possibility, mentioned by Wilson (1939), that 'some change in the tissue destined to become meristematic might precede appearance of the phellogen, and that such a change might check canker extension'.

In the whole of the foregoing work it will have been apparent that artificial inoculations failed to produce any very bad cankers extending many feet in length or fatally girdling the stem, such as are characteristic of the disease in its worst form. The descriptions given of the mode of invasion of the pathogen and of the defence mechanism of the two varieties of host are therefore to be regarded as illustrative of the early stages of infection, or, more generally, of those years in which no severe outbreak of bacterial canker occurs.

I wish to express my thanks to the Agricultural Research Council for a personal grant, to the University College Hospital Medical School and East Malling Research Station for hospitality, to Dr E. E. Wilson of the University of California for advice on sectioning technique, and to Dr H. B. S. Montgomery of East Malling Research Station for field collaboration in the inoculation experiments.

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Certain aspects of resistance of plum trees to bacterial canker

Part III. The action of cell-free filtrates of *Pseudomonas mors-prunorum* Wormald and related phyto-pathogenic bacteria on plum trees

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(With 2 Text-figures)

A technique for introducing 2–3 c.c. liquid into the bark of plum trees is described. Parallel injections with dyestuff throughout the spring failed to afford a reliable index of the invasive powers of the various test fluids introduced in this manner.

A susceptible variety of plum (Giant Prune) showed greater injury by cell-free filtrates of *Ps. mors-prunorum* than did a resistant variety (Warwickshire Drooper), which showed negligible injury. The greatest injuries were caused by filtrates of cultures 5 weeks old, and over, especially when concentrated.

Of other bacteria tested *Ps. prunicola*, *Ps. syringae* strains, *Syringa* 3, Ap. No. 1, Ap. No. 2, and *Bact. pruni*, yielded damaging filtrates, whereas *Ps. syringae*, strains pear 6 and 7, *Ps. tabaci*, *Ps. fluorescens*, *Ps. marginalis*, *Ps. phaseolicola*, *Ps. tumefaciens*, *Ps. pisi* and *Ps. cerasi* failed to do so.

Some evidence is advanced for the view that the deleterious activity of *Ps. mors-prunorum* may be due in part to an endotoxin of protein nature obtainable from the dried bacterial cells by acetic acid extraction.

INTRODUCTION

The histological examination of bacterial cankers described by Erikson (1945*b*) revealed that *Pseudomonas mors-prunorum* Wormald invaded plum bark intercellularly, and entered the cells only when they were already isolated and disintegrating. This fact suggests that the progress of the bacteria was assisted by the production of a substance or substances toxic to the host tissue. The experiments to be described were designed to test whether such a toxin is produced exocellularly by *Ps. mors-prunorum* growing on bark extracts or synthetic media, whether this toxin is produced specifically by *Ps. mors-prunorum*, and whether a susceptible variety of plum is damaged more readily than a resistant variety by the action of the toxin.

EXPERIMENTAL MATERIAL

Eighty 3-year-old plum trees were planted 2 ft. apart in rows 6 ft. apart, with the initial aim of providing biological controls for the chemical examinations to be made at those seasons (autumn and spring) that are the contrasting periods for bacterial invasion (Wormald, 1931). These trees were arranged in groups of five, groups of a resistant variety (Warwickshire Drooper) being randomized with groups of a susceptible one (Giant Prune). Use was made of a further set of 100 Giant Prunes planted in rows alongside. No more Warwickshire Droopers were available. The Prunes were raised on Myrobolan Seedling stocks and the Droopers were on their own roots.

The micro-organisms employed in these tests were as follows:

Ps. mors-prunorum Wormald; strain A61.

Ps. prunicola Wormald; strain A46.

Ps. syringae van Hall; strains Ap. No. 1, Ap. No. 2, *Syringa* 3, pear 6, and pear 7.

Ps. fluorescens-liquefaciens, *Ps. pyocyaneus*, *Bact. tumefaciens* 'tomato' strain, *Ps. pisi*, *Ps. tabaci*, *Ps. cerasi*, *Ps. phaseolicola*, *Ps. marginalis* and *Bact. pruni*.

The description and origin of these cultures is given in Part I of this series (Erikson, 1945*a*).

* Special Research Officer, Agricultural Research Council.

Preparation of bark extracts, culture media and filtrates

Bark extracts were prepared in the first instance in spring 1942 by Drs. Harington & Pitt-Rivers, from whose unpublished Report on the earlier part of this work we are permitted to quote the following method: 'About 1 kg. fresh bark was shredded (by means of a rapidly rotating wire brush) directly into ether; the ether extraction was repeated three times to remove the last traces of chlorophyll and the other ether-extractable material, and the residue was air-dried. The yield was 620 g., of which 300 g. were hydrolysed at 95° in 3 l. *N*/2 sulphuric acid for 6 hr., after which the reducing value of the hydrolysate determined by Benedict titrations remained constant. The liquor was separated from the shreds and divided into two portions: one was neutralized with sodium hydroxide and the other with baryta. Barium sulphate was removed from the latter and the two solutions were concentrated at a low temperature (40°); their sugar contents were determined as apparent glucose and a sulphate determination was also made on the soda-neutralized portion to avoid the introduction of excessive salt into the culture medium.'

A further supply of extracts was prepared the next spring by the same method.*

Each extract was buffered to *p*H 7·0 and adjusted to contain approximately 1% apparent glucose. (It was later found by Harington & Pitt-Rivers that arabinose formed a high percentage of the total reducing sugar present.) They were sterilized by filtration and provided good nutritive media for all bacteria tested. The four bark extract media were as follows:

S Na = NaOH neutralized acid hydrolysate of susceptible bark.
R Na = NaOH neutralized acid hydrolysate of resistant bark.
S Ba = $Ba(OH)_2$ neutralized acid hydrolysate of susceptible bark.
R Ba = $Ba(OH)_2$ neutralized acid hydrolysate of resistant bark.

A standard synthetic medium was also used containing sodium ammonium hydrogen phosphate (microcosmic salt) or ammonium phosphate as the sole source of nitrogen, and glucose as the carbon source. In a few instances sucrose, or later fructose, was used instead of glucose, but in general sucrose was not used, to avoid possible complications from the bacterial polysaccharide produced by the levan-forming organisms.

The cultures were grown in flasks at 24° C. for periods ranging from 4 to 12 weeks, except in preliminary experiments when younger cultures were also used. They were all tested for viability and purity before being filtered and, while all were viable up to 6–7 weeks, *Ps. mors-prunorum* and *Ps. prunicola* possessed superior longevity to many of the other varieties, which frequently died in the ninth or tenth week.

The filtrates were prepared by passing the centrifuged cultures through a Seitz filter, and tested for sterility before being put into the tree. They ranged from *p*H 5 to 6, and comparably acidified controls were therefore injected in parallel with the filtrates.

Technique

In the preliminary tests, designed to see if bacteria-free cultures of the extracts had any observable effects, the filtrates were put into the under-layers of the bark and the outer layers of the wood by a hole, produced by running a needle tangentially under the bark, into one end of which was inserted the bent, finely drawn end of a small glass tube (Fig. 1). The filtrate was placed in the glass tube with care to avoid air-locks, and the seepage of the liquid from the open end of the needle track indicated that it was reaching the tissues.

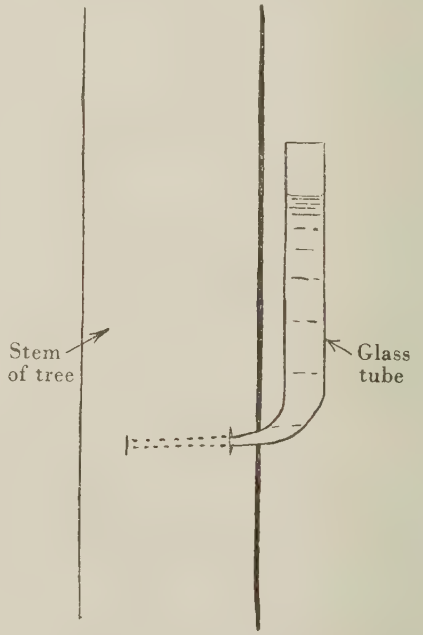

Fig. 1. Bent glass-tube method of injecting filtrates.

As a rule four such 'injections' were made into each tree using the same filtrate, with sterile unsown media of identical composition as controls on a similar tree, and the method proved satisfactory for broad qualitative results. It was, however, not easy to estimate accurately the amount of fluid actually reaching the cells by this method because, although the tubes had an average capacity of 1 c.c., it was

* The authors are indebted to Dr. H. Shaw of E.M.R.S. for assistance in preparing these extracts.

found impossible to fix them so that the liquid seeped through the hole in the bark at the same rate. This lack of uniformity was in part due to variability in the bend of the tubes, but more often the result of variability of the bark itself. There was also both a seasonal and varietal difference in the brittleness of the bark. Most liquid was taken up by the host tissues where the loss due to seepage through the exit hole was least.

When more accurate methods became necessary three methods were compared using a 1% solution of *patent blue* dye to indicate the area affected, as

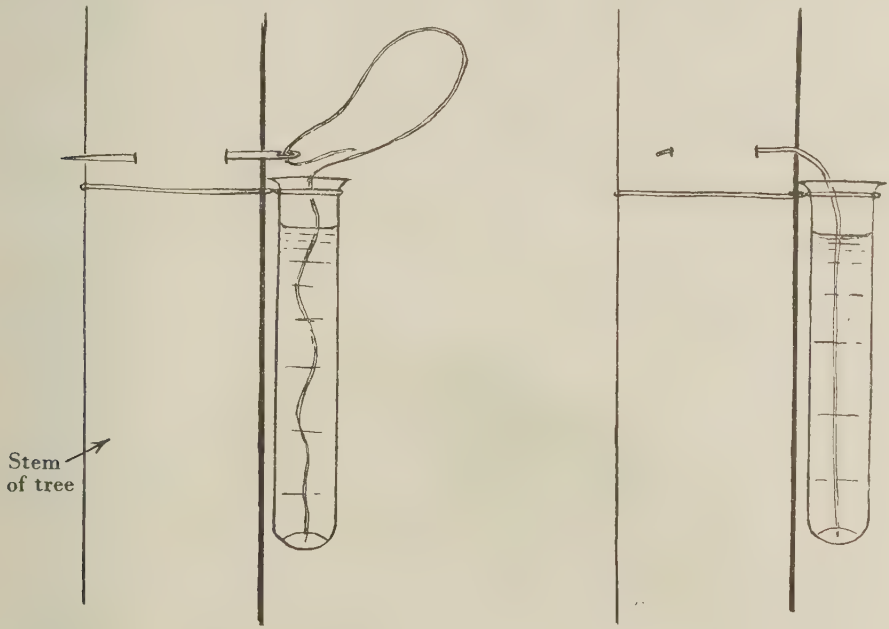

Fig. 2. Tube and wick method of injecting filtrates.

follows: (1) by bent tube as previously, (2) by inserting a swab of cotton-wool saturated with the dye in a deep cut made into the bark, (3) by a tube and wick method. In the last method, a small test-tube holding 3 c.c. of the dye was hung on the stem of the tree and the liquid was carried to the bark by a wick made of a woollen thread, attached to the bottom of the tube by a drop of paraffin wax. The thread was drawn under the bark by means of a needle pushed through the bark tangentially, the protruding end of the thread being cut off close to the exit hole (Fig. 2).

This comparison of methods was made in February 1943, and within 3 days it was found that method 3 was the most effective, method 2 being least so. In method 2 the dye tended to reach the underlying wood rather than the bark and the very marked wounding was undesirable. Method 1 dyed a narrow area of bark extending about 10 mm. from the site of injection, with a few streaks in the wood from 20 to 50 mm. in length. Method 3 gave more uniform results, staining the bark chiefly in an irregular oval patch averaging about 30 × 10 mm.

This tube and wick method appeared to have the advantage also of supplying more liquid (approx. 3 c.c./tube), which was not wasted but was taken up directly by the tissues in contact with the wick in the needle track. The needle track itself caused negligible mechanical injury, thus making all measurements of injury more accurate. The loss due to evaporation from the tube with wick was found to be less than half the volume of the tube in 3 days, even under conditions of rapid evaporation. Under such conditions, therefore, the tissues ought to receive at least 1·5 c.c. of the liquid, appreciably more than by method 1. All these methods were, however, subject to some dilution by rain. Method 3 was selected for all the later experiments.

During February and the first half of March 1943, using method 3, the bark was stained to a similar extent by all the dye controls, indicating in general a greater movement through the tissues than that of the various filtrates as shown by the subsequent browning. But from late March to early May some filtrates produced a wider effect compared with the results of corresponding dye controls, which remained constant or slightly decreased as the season progressed. The extent of the dye-stain, therefore, gives no reliable index of the infiltration of other

substances under the seasonal changes of the host. For comparison, inoculations with aqueous suspensions of 2-day-old cultures of *Ps. mors-prunorum* were made with a hypodermic needle.

Results

Injection method 1. The results of experiments made with the bent-tube technique, covering the period from July to late November 1942, were too variable for presentation in detail, but the following conclusions may be drawn from the 386 injections.

All filtrates from cultures of *Ps. mors-prunorum*, 2 weeks old or less, irrespective of the medium on which they were grown, produced only very slight injury to the host tissues in excess of that caused by the control injection. This injury was most marked with two filtrates from 2-week-old cultures on *S* Na and *R* Na respectively, injected on 2 Oct. 1942. Filtrates from older cultures of *Ps. mors-prunorum*, 5, 6 and 7 weeks old on bark extracts, usually caused more browning than the controls. One similar set of filtrates from 5-week-old cultures of *Ps. fluorescens* caused negligible browning.

An insufficient number of comparisons was made between the susceptible and resistant varieties to draw any definite conclusions, but, in general, the resistant variety was the less affected.

Injection method 3. The experiments carried out with this method gave results that could be recorded numerically. The injuries to the host were classified as follows:

0 = mechanical injury only.
1 = very slight browning, less than 2 mm. mean diam.
3·5 = browning in extent 3·5 ± 1·5 mm. mean diam.
7·5 = browning in extent 7·5 ± 2·5 mm. mean diam.
15 = browning in extent 15 ± 5·0 mm. mean diam.

Over 20 mm. is estimated to the nearest 10 mm. These measurements refer to the injuries to the bark tissues, and as a rule a discoloured area of similar extent was present also in the wood. In several trees lines of discoloration extended to a much greater distance in the wood.

Phellogen round the damaged tissue was noted towards the end of April, and in the first week of May clearly defined periderm was found in both varieties of plum tree. As there was no difference between the two hosts in this respect no mention of it is made in the tables.

Table 1. *Size of lesions produced in bark by cell-free filtrates of cultures of* Ps. mors-prunorum (*strain* A61) *on various media, and by living cultures compared with the spread of similar injections of dye* (*mean diam. in mm.*)

Medium	Age of culture weeks	Date of injection 1943	Examined after weeks	Susceptible		Resistant		Susceptible	
				Filtrate	Control	Filtrate	Control	Culture	Dye
S Na	6	5 Feb.	2	7·5	1·0	3·5	0·0	15·0	30·0
			8	15·0	3·5	1·0	0·0	25·0	30·0
Glucose microcosmic salt	6	3 Mar.	3	7·5	3·5	3·5	0·0	—	7·5
			5	7·5	3·5	3·5	3·5	30·0	20·0
R Ba	5	16 Mar.	3	20·0	3·5	5·0	1·0	3·5	7·5
			7	15·0	1·0	1·0	0·0	7·5	15·0
			9	20·0	3·5	3·5	0·0	3·5	7·5
Glucose microcosmic salt	8	14 Apr.	2	15·0	3·5	1·0	1·0	—	15·0
			7	7·5	0·0	0·0	0·0	—	3·5
Fructose ammonium phosphate	5	28 May	3	15·0	7·5	3·5	3·5	—	7·5
			8	15·0	3·5	3·5	3·5	—	7·5
R Na	6	8 June	16	30·0	7·5	7·5	3·5	—	—
S Ba	6	9 July	4	7·5	3·5	7·5	3·5	—	—
			13	7·5	3·5	3·5	3·5	—	—
R Na	9	6 Aug.	9	15·0	7·5	15·0	7·5	—	—
R Ba	9	6 Aug.	9	20·0	7·5	7·5	3·5	—	—
S Ba	5	20 Aug.	8	7·5	3·5	7·5	7·5	—	—
			Average	13·7	3·9	4·6	2·4	14·1	13·7

Table 1 shows the response to cell-free filtrates of *Ps. mors-prunorum*, the effect being produced with filtrates from synthetic and bark extract cultures. The averages show that injury occurred on the susceptible variety but was negligible on the resistant one. In Table 2 the summarized results of the bark extracts, taken from Table 1, show a tendency for cultures on the resistant bark extracts to provide more toxic filtrates than those on the susceptible ones.

Of the other organisms tested the *Ps. syringae* strains pear 6 and 7, *Ps. tabaci*, *Ps. fluorescens*, *Ps. marginalis*, *Ps. phaseolicola*, *Ps. tumefaciens*, *Ps. pisi* and *Ps. cerasi* all failed to yield filtrates more injurious

than the control. Filtrates of *Ps. syringae*, strain *Syringa* 3, caused much injury on the susceptible variety (Table 3).

The action of the other cultures yielding toxic filtrates is shown in Table 4: *Ps. prunicola* (A46) was the most damaging of these.

In two instances exceptionally severe damage (lesions up to 50 mm.) followed the injection of a filtrate. In one of these, the filtrate was obtained from a 3-month-old culture of *Ps. mors-prunorum* on sucrose ammonium phosphate medium, stored for a further 2 months in the cold room before filtering. This filtrate was concentrated to one-fourth of its volume before use in February. Another damaging filtrate was obtained from a 2-week-old culture of *Ps. syringae* Ap. No. 1, grown on glucose ammonium phosphate medium. It was concentrated to one-half of its volume before use in March. The control injection for each was the unsown medium at normal strength comparably acidified (*p*H 5) and, therefore, it is not possible to say with certainty that the effect was due to a toxin produced by the organism and not to the concentration of the salts in the medium. However, as both filtrates were from ammonium phosphate media, there were no sodium ions and the amount of phosphate present, even when multiplied by four, would not equal that in a heavily buffered microcosmic salt medium which was injected in early March without any appreciable reaction. Quadrupling the sulphate present in the medium brings it to 0·08%, and a 1% solution of sodium sulphate has been introduced into the trees without causing any marked injury. Furthermore, a four times concentrated medium was injected in May without causing injury. It would seem, therefore, that the result is not attributable to the hypertonicity of the solution alone, although the possibility of the toxicity of the inorganic salts in this strength when combined with some one or several bacterial metabolites should not be overlooked. Samples from the necrotic areas of these trees were found to be sterile.

TABLE 2. *Average size of lesion* (*Table* 1) *for each bark extract* (*mean diam. in mm.*)

	Susceptible		Resistant	
	Filtrate	Control	Filtrate	Control
S Na	11·25	2·25	2·25	0·0
R Na	22·5	7·5	11·25	5·5
S Ba	7·5	3·5	6·2	4·8
R Ba	19·0	4·0	4·25	1·1

TABLE 3. *Size of lesions produced by cell-free filtrates of cultures of* Ps. syringae *strain* Syringa 3 (*mean diam. in mm.*)

Medium	Age of culture weeks	Date of injection 1943	Examined after weeks	Susceptible	
				Filtrate	Control
Synthetic glucose microcosmic salt	6	3 Mar.	3	20·0	3·5
			5	60·0	3·5
Do.	11	9 Apr.	3	7·5	0·0
			7	3·5	1·0
Glucose ammonium phosphate	7	20 May	2	7·5	3·5
Fructose ammonium phosphate	5	28 May	3	20·0	7·5
			8	15·0	3·5
S Ba	6	9 July	4	15·0	3·5
			13	7·5	3·5
R Ba	9	5 Aug.	9	15·0	7·5
S Ba	5	20 Aug.	8	7·5	3·5
Fructose microcosmic salt	11	20 Aug.	8	30·0	3·5
			Average	17·4	3·7

TABLE 4. *Size of lesions produced in susceptible host by cell-free filtrates, summarized irrespective of culture media* (*mean diam. in mm.*)

Month of injection	Control	*Ps. syringae* Ap. No. 1	*Ps. syringae* Ap. No. 2	*Ps. prunicola* A46	*Ps. pruni*
Mar.	3·5	—	7·5	15·0	—
	3·5	—	—	20·0	—
Apr.	0·0	3·5	—	—	7·5
	1·0	1·0	—	—	15·0
Apr.	3·5	—	—	0·0	7·5
	0·0	—	—	3·5	1·0
May	7·5	7·5	7·5	15·0	—
	3·5	7·5	3·5	20·0	—
June	7·5	—	—	15·0	—
July	3·5	15·0	—	15·0	7·5
	3·5	3·5	—	7·5	7·5
Aug.	3·5	15·0	15·0	20·0	—
	3·5	7·5	—	—	
	7·5	15·0	—	—	
Average	3·7	8·4	8·4	13·1	7·7

Attempted extraction of endotoxins from dried bacterial cells of *Ps. mors-prunorum*

Since filtrates of aged cultures caused in general a more marked response in the susceptible host than filtrates of young cultures, it seemed that the effect produced might be due, at least in part, to a substance or substances resulting from bacterial autolyses, and the following experiments were made.

Ps. mors-prunorum (strain A61) was grown for 1–3 weeks in a liquid synthetic glucose-ammonium phosphate medium to avoid possible agar contamination of the bacterial polysaccharide (Freeman *et al.* 1940). The bacterial bodies were collected by simple centrifugation, washed and dried, with a yield of 0·77 g. from 1 l. of culture. This yield was improved to 5·25 g. from 6 l. by passing through a Laval Separator scraping the bacterial mass from the bowl and drying.

Acetic acid extraction of the dried bodies by the method of Freeman & Anderson (1941) produced a liquid readily cleared by spinning which, after dialysing against water in collodion sacs, was neutral in reaction and gave positive arginine, Esbach, Biuret and Molisch tests and negative Fehling's reaction. This indicated the absence of any of the glucose supplied in the medium and the presence of protein and polysaccharide which could be derived only from the bacterial bodies themselves, since no organic nitrogen or colloidal carbohydrate was present in the medium.

The acetic acid extract when treated with alcohol or acetone yielded a copious precipitate. The dialysate of a trichloro-acetic acid extract (Boivin *et al.* 1935*b*), which gave negative protein and positive carbohydrate reactions, yielded a much smaller acetone precipitate.

The various preparations in neutral aqueous solution were injected into susceptible and resistant trees during the period Mar.–May 1943. An outstanding result was given by the Seitz-filtered, unheated, unprecipitated dialysate of the acetic acid extract in the susceptible host—a lesion in the bark of 190 mm. in one instance, 80 and 50 mm. in others, as compared with 15 mm. in the resistant tree, and 15 and 20 mm. for the living culture and dye controls respectively in the susceptible host. The less marked results given by the heated dialysate or precipitate therefrom suggests that some denaturation of the protein constituents may have occurred during such treatment. Trichloro-acetic acid extracts had a negligible effect, which points to the probability of the toxicity being caused by protein rather than by polysaccharide constituents of the bacterial bodies.

General discussion

From Part I of the present series (Erikson, 1945*a*) it may be concluded that as *Ps. mors-prunorum* can utilize a very wide range of carbohydrate and nitrogen sources it is improbable that the resistance of certain plum varieties to bacterial canker can be due basically to a relative shortage of essential food materials. It is, however, worth noting that this pathogen cannot utilize starch, cellulose, or any polysaccharide tested except pectin. It was found to tolerate most of the known or probable chemical constituents of plum bark at comparable concentrations, the sole exceptions being tannins and phenols. Comparative information, quantitative and qualitative, on the chemical composition of the susceptible and resistant types of plum tree is clearly required before further progress can be made along this line of approach to the problem.

The histological data submitted in Part II (Erikson, 1945*b*) show that the observed invasion of the tissues by the bacteria was intercellular, and it therefore appears unlikely that any delicate symbiotic balance is involved. The details of invasion were exactly the same for both the resistant and the susceptible variety, except that, in the former, the invasion progressed to a less degree and periderm formation shut off the invaded tissue at a very early stage. The organism was found to be capable of living in both hosts for considerable periods without much involvement of tissue, almost as a saprophyte tolerated within a well-barricaded area, but there was no evidence concerning the factor or factors that sometimes can cause the organism to spread so greatly and destroy so much tissue in the susceptible host.

That disintegrating cells were seen in the sectioned material before any bacterial bodies had entered the cells suggested the production of a toxin by the organism capable of penetrating and killing living cells in advance of actual penetration by the bacteria. The injections with cell-free filtrates, described in this paper, support this hypothesis, since these filtrates, introduced into bark tissues, produced injury very similar to that caused by the organism itself, and histological examination revealed a similar progression of the injury. The reaction of the resistant and susceptible plum varieties to the filtrate appeared to be identical with their reaction towards the organism. As toxic filtrates were produced from cultures on bark extracts from both types of variety and on synthetic media, it would appear that resistance is more likely to lie in the reaction of the host to the toxin than in a limitation in the production of toxin due to the presence of an inhibiting substance or substances or to the lack of some essential constituent for the growth and parasitic development of the organism.

The development of this toxin is believed to be specific to organisms closely related to *Ps. mors-prunorum*, since comparable cultures of other parasitic and saprophytic organisms failed to yield toxic filtrates. Because toxic filtrates were not obtained from young vigorously growing cultures (age less than 2 weeks), but only from aged ones, a search was

made for an endotoxin that might be liberated by the autolysis of the organism, and this revealed a toxin of protein nature obtainable from the dried bacterial cells by acetic acid extraction. This compares with the results of Boivin *et al.* (1935*a*), who claimed to have obtained from *Bact. tumefaciens* by trichloro-acetic acid extraction an antigenic complex which, when injected into young stems of *Helianthus annuus*, produced tumours comparable with those produced by the living organism. In the present case, however, the extraction with acetic acid was much more effective than with trichloro-acetic acid. No accurate knowledge of the antigenic constitution of *Ps. mors-prunorum* is yet available, and the serological properties cannot, therefore, be correlated with pathogenicity or variations in virulence.

It is suggested that the progression of the canker caused by *Ps. mors-prunorum* in plum trees may be due to a toxin liberated by the autolysis of the bacterial bodies, possibly in conjunction with metabolites produced from some constituent of the host tissues. Some varieties of plum may be resistant to this invasion because either they are not affected by the toxin or they speedily delimit the spread of the bacteria or toxin by a mechanical or chemical barrier.

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On the spread of crinkle in Royal Sovereign strawberries in south-west England

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(With 4 Text-figures)

Observations at periodic intervals on marked plants in strawberry plantations in south Devon showed that the maximum appearance of crinkle symptoms occurs in June and July, in which months roguing and inspections should be completed. The data suggest that infection in summer by apterous aphides passing on to neighbouring plants is probably the principal cause of spread.

INTRODUCTION

The Royal Sovereign variety of strawberry is one of the most susceptible to the virus diseases yellow edge and crinkle. Both occur in Devon and Cornwall but crinkle plays much the larger part in the degeneration of strawberry stocks and is the main factor shortening the life of commercial strawberry beds in this area. In the Tamar Valley in particular, after about 1920, Royal Sovereign beds could rarely be kept profitably for more than 3 years. Amongst growers this was ascribed to the patch disease from the appearance of affected plantations. Diseased plants became mori-

bund owing to crinkle added to the effects of adverse soil conditions. In the first investigations of this trouble in 1926 the non-committal name of leaf curl was used (Hodson & Beaumont, 1928). The name crinkle was first used by Zeller & Vaughan (1932) in America. Ogilvie *et al.* (1934) described the occurrence of the disease in England in 1928 and stated that the symptoms were identical with the American disease. In 1934 the disease was observed by Harris (1937) at East Malling and the common identity and virus origin of the diseases from these two English sources were established.

Earlier observations in the Tamar Valley

In order to see how crinkle (then known as leaf curl) spread, observations were made on batches of plants selected as representative of the plantations. These observation plots were either rectangular (comprising several rows), as in quadrats D and E reported below, or consisted of a series of plants in line across the plantation (line transect F). Examples of these observations are given in Table 1.

In all three examples the maximum development of crinkle occurred in June and July. The apparent decrease in the autumn and winter was due to the absence of symptoms which are not so easily seen on the old leaves. When the new leaf growth appeared the following spring the symptoms reappeared on still larger numbers of plants. As crinkle is transferred by the strawberry aphis (*Capitophorus fragariae* Theobald), the maximum populations of which occur in the early summer (Hodson, 1937; Thomas & Jacob, 1940), it seems probable that the spread took place in the summer months and that many of the infected plants did not show the symptoms until the new growth began in the following spring.

Observations 1939–40 at Dartington, south Devon

Conditions in the Tamar Valley have become very adverse to the strawberry plant owing to the larger number of seasons of continuous strawberry cultivation and to the increasing difficulty in getting organic manure. The occurrence of the disease at Dartington, south Devon, in plantations on new ground, which was well manured, gave the opportunity of carrying out detailed observations to ascertain how far spread might take place in good soil conditions well isolated from other strawberry plantations. [Reference may be made to *Soft Fruit Growing* by Raymond Bush (Penguin Books), revised edition, 1944; Pls. XI and XII are photographs of the field containing the plants on which the observations in this paper were made.] The plots were larger than in the Tamar Valley but in the same quadrat form. Unfortunately, the war ended the observations in 1940 but the incomplete results are of interest.

The method of making and recording the observations was as follows:

The quadrats were marked by means of stakes and a plan of each quadrat was made on squared paper, one square being allocated to each plant. Gaps where plants had been removed prior to the commencement of the observations were left blank, and these squares remained blank throughout the series of plans; subsequent deaths and removals of plants were indicated by means of a cross in the appropriate squares.

At each observation a separate plan was made of each quadrat, the squares representing the plants being marked to indicate degrees of crinkle disease and healthy plants. In this way a permanent picture record was obtained of the progress of the disease. By transferring the squares relating to any particular type of plant on one plan to tracing paper, the latter could then be applied to the next plan in the series. In this way a complete analysis of all the changes

Table 1. *Increase of crinkle in observation plots during* 1923 *and* 1925

D. Planted autumn 1923

Date	Healthy		Crinkle	Dead or dying
19 May 1926	30		3	0
1 July ,,	29		3	1
23 Oct. ,,	27		3	3
3 Feb. 1927	21		1	0
28 Mar. ,,	—	only	8	8
6 May ,,	—	new	10	1
7 June ,,	—	runners	3	2

Nearly half the plants died in the winter of 1926–27. The bed was destroyed 13 July 1927.

E. Planted autumn 1925

1926. All 29 plants healthy

Date	Crinkle
2 Mar. 1927	3
28 Mar. ,,	3
6 May ,,	8
7 June ,,	8
13 July ,,	All slight crinkle
29 Aug. ,,	All definite crinkle
Mar. 1928	Many runners planted to fill the gaps
June 1929	Bed destroyed

F. Planted autumn 1923

Date	Healthy	Crinkle	Dead or dying
19 May 1926	34	18	0
2 July ,,	31	21	0
23 Oct. ,,	30	4	18
3 Feb. 1927	17	15	20
28 Mar. ,,	15	9	28
6 May ,,	16	18	18
7 June ,,	15	17	20

which had taken place in individual plants from one observation date to another could readily be made.

Quadrats A and B were in one field of about 6 acres, A being the part planted in 1937 and B in the larger part planted in 1938. All the plants came from the same original source grown elsewhere on the farm.

At the time of the first observation, quadrat A consisted of 188 plants and 32 gaps. These gaps were probably occupied by severe crinkle plants in 1938, and are separately indicated in the diagrams. Table 2 gives the percentage of the original number (188) showing disease on the dates given (Fig. 1).

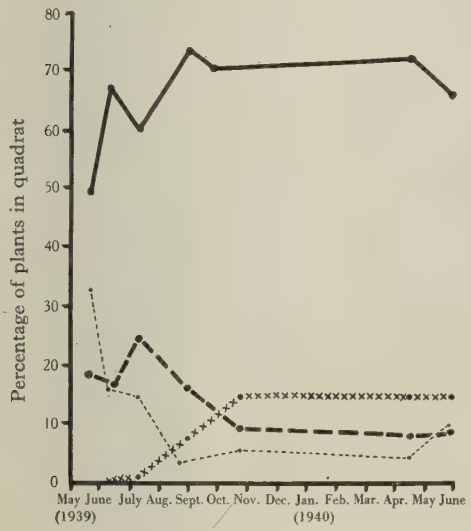


Fig. 1. Quadrat A.

Healthy plants. — — — 'Severe crinkle' plants.
×××××××× Dead plants. --------- 'Slight crinkle' plants.

TABLE 2. *Percentage of crinkle plants observed in quadrat A*

Quadrat A. (2-year-old plants)

Date	Healthy %	Crinkle (%) Slight	Crinkle (%) Severe	Dead %
18 May 1939	15·4	66·4	18·2	0
9 June ,,	67·2	16·0	16·8	0
4 July ,,	60·1	14·9	24·5	0·5
30 Aug. ,,	73·4	3·2	16·0	7·4
25 Oct. ,,	70·8	5·3	9·1	14·8
15 Apr. 1940	72·9	4·3	8·0	14·8
27 Apr. ,,	66·5	9·6	9·1	14·8

Quadrat B. (1-year-old plants) comprised 246 plants and 28 gaps. Table 3 gives the percentage of the original number (246) showing disease on the dates given (Fig. 2).

The crinkle plants were divided into two classes, (*a*) slight and (*b*) severe. The former referred to plants with spots on the leaves and showing the characteristic distortion of some or all of the young leaves, but not showing any reduction in size. Badly affected plants, in addition to these symptoms, had shortened petioles giving a characteristic flattened appearance to the plant which was often seriously reduced in size (cf. Ogilvie *et al.* 1934).

As the spring of 1939 was a late one the plants were only just expanding their young leaves at the time of the first observation, and the plants affected with mild crinkle only (Harris, 1937) were included

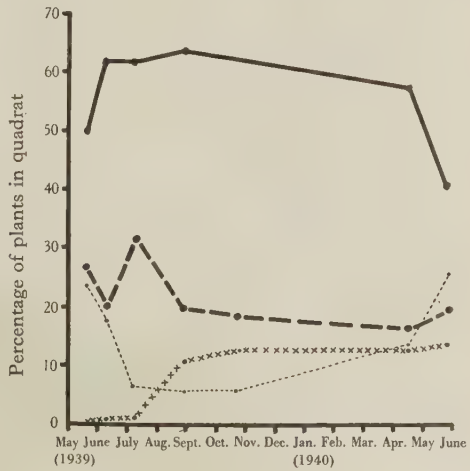


Fig. 2. Quadrat B.

Healthy plants. — — — 'Severe crinkle' plants.
×××××××× Dead plants. --------- 'Slight crinkle' plants.

TABLE 3. *Percentage of crinkle observed in quadrat B*

Date	Healthy %	Crinkle (%) Slight	Crinkle (%) Severe	Dead %
18 May 1939	21·0	52·3	26·7	0
9 June ,,	61·9	17·6	20·0	0·5
4 July ,,	61·9	6·1	31·3	0·8
30 Aug. ,,	63·9	5·8	19·9	10·4
25 Oct. ,,	63·3	5·8	18·7	12·2
15 Apr. 1940	57·6	13·6	16·2	12·6
27 May ,,	40·9	25·6	19·9	13·6

in the first class. When it was found in July that every plant showed mild crinkle no further notice was taken of such symptoms. The apparently large increase in healthy plants in June is thus due to the recognition of mild crinkle and its inclusion in the healthy plants.

Both quadrats showed the maximum appearance of true crinkle in early July. Roguing of strawberry plantations should therefore be carried out in June and July. In an early season crinkle will be visible in May, in which case most of the roguing may be finished by the end of June.

At the observation in early July there was a change over from slight to severe symptoms which follows naturally from the growth to maturity of the plant, allowing it to show the full symptoms. After this date diminution in the number of plants showing bad crinkle is due to the fact that a large proportion of them die. Almost without exception the plants of the last column in the tables had previously shown severe crinkle. This is clearly shown by the diagrammatic record of plots (Figs. 3, 4).

In the following spring there was a marked increase in the amount of slight crinkle in the younger plants (quadrat B), and a slight increase in those in quadrat A. This suggests, as was observed from the early Tamar Valley data, that plants may become infected in the early autumn and remain apparently healthy until the new growth starts the following spring. If this be the case it further confirms the necessity of carrying out roguing and inspections during the period May–July.

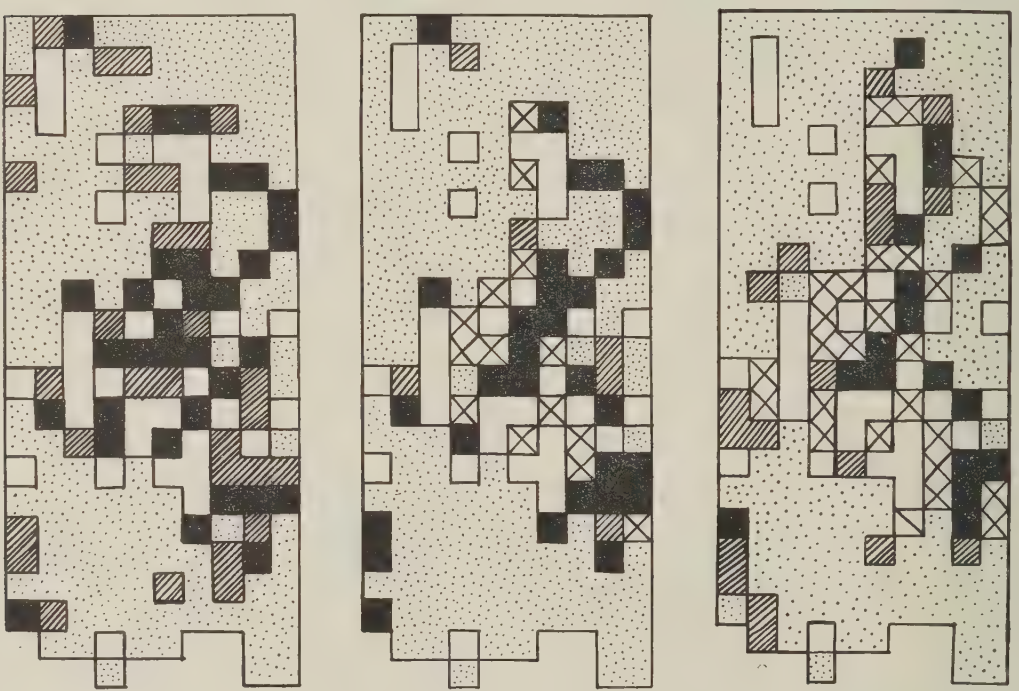


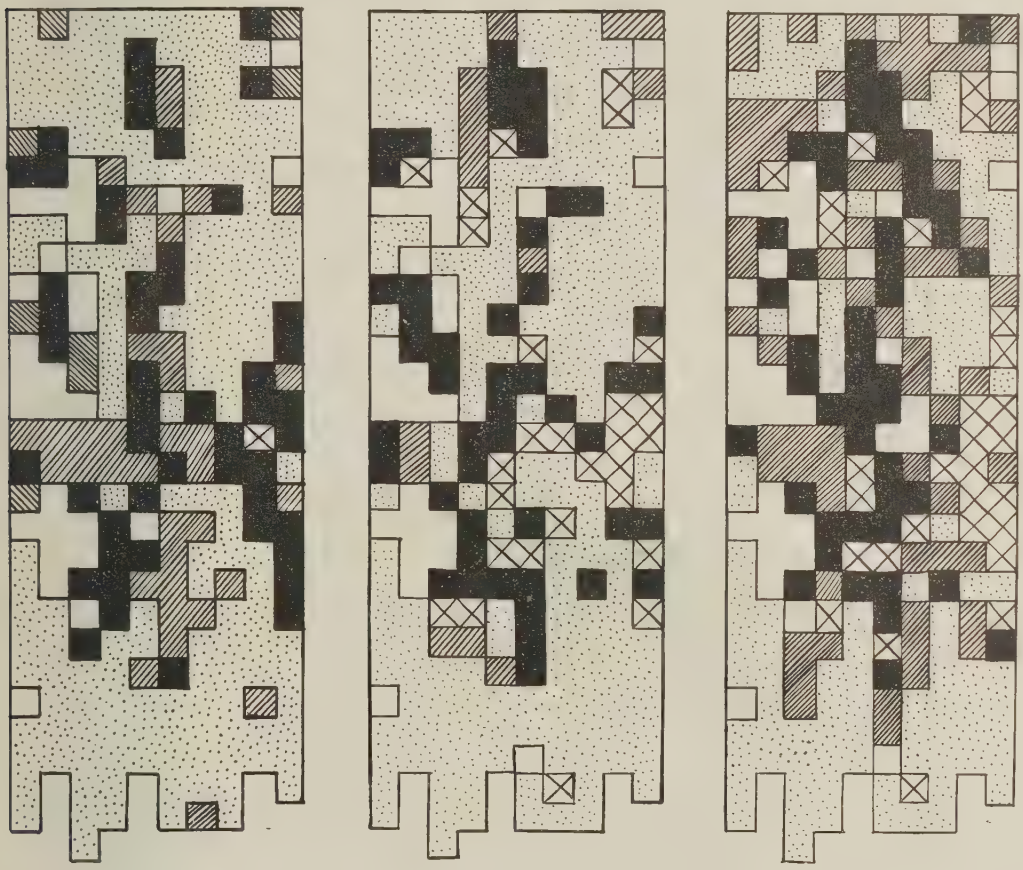
Fig. 3. Quadrat A.

The rise in the percentage of healthy plants at the end of August in quadrat A is due to twenty-five slight crinkle plants becoming apparently healthy (cf. Fig. 3). This is part of the general improvement in the growth of strawberry plants that takes place during the summer months; it was observed that many quite bad crinkle plants showed less severe symptoms in August. As several of the healthy plants showed crinkle again the following April it is probable that the recovery was only apparent. This is another reason for completing crinkle inspection work in July.

The spread of crinkle did not take place in any

particular direction but was always to contiguous plants. This is clearly shown by Figs. 3 and 4, which record the symptoms on each plant on the dates given. This suggests that the spread of crinkle takes place slowly by apterous aphides crawling on to neighbouring plants.

Both quadrats showed a certain amount of yellow edge at each observation. The maximum number in quadrat A was 15 plants (8%) on 9 June, and in quadrat B 42 plants (13%) on 4 July. The majority of them were affected with crinkle as well and a large proportion of these plants died.



9 June 1939. 30 Aug. 1939. 27 May 1940.

Fig. 4. Quadrat B.

Key as shown in Fig. 3.

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The control of the sheep-tick (*Ixodes ricinus* L.) by treatment of farm stock

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The sheep tick is practically confined to the rough hill-grazings in Britain where the chief domestic hosts are sheep and cattle. The practical difficulties of treating sheep, lambs and cattle are outlined. Sheep with lamb-at-foot, and suckling Galloway and Kyloe cows unused to handling, are the biggest problems.

Research on anti-tick materials having been done chiefly by commercial firms, public knowledge is very scanty. Thirty-nine experimental and commercial anti-tick preparations were tested on sheep, lambs and cattle.

A sheep dip must be safe, easily mixed, have lasting effect, be capable of dealing with all or most of the ecto-parasites present and reasonably cheap. So far no experimental dip has surpassed a commercial product comprised of sodium arsenite, ground derris root, cresols and woolfat. In the case of the female tick, with 30 sec. immersion this dip kills all ticks attached at time of dipping and keeps sheep free of ticks for 7–10 days, while reinfestation is not complete until 3–4 weeks after dipping. Derris is chiefly responsible for duration of residual effect in this dip. Unlike arsenic, derris kills a proportion of ticks before they can attach and thus lessens tick-worry and the chances of disease transmission. The dip has no ill-effects on sheep.

The present method of assessment by tick counting does not quite show the full lethal effect of dips. No improvement in the method seems possible.

Changes in the bath fluid and in residual protection for sheep were studied at different stages of normal dippings (*c.* 200–300 sheep) in spring. Herdwick ewes and Black-faced yearlings each finally carry away about ½ gal. (2·28 l.) of dip fluid in their fleeces. A large quantity of foreign solid matter is added to the bath by sheep as dipping progresses; arsenic content remains unchanged while derris is somewhat reduced. The ground derris root particles are thought to be trapped in the meshes of the fleece as excess dip fluid runs back to the bath. Surface tension increases from about 48 to 50 dynes/cm. Residual protection for sheep against ticks lessens as dipping progresses, probably due to progressive reduction of derris in the bath. On the average, provided each sheep gets its share, the dip could furnish each sheep with not more than 0·08 oz. (2·27 g.) ground derris root, 0·18 oz. (5·10 g.) arsenic (as As_2O_3), and woolgrease at the rate of 10–17% (0·64–1·12 oz. or 18·14–31·75 g.) of the weight naturally present in the fleece (6·40 oz. or 181·44 g.). The dip is effective against all ecto-parasites except blowfly larvae.

Light smearing with vaseline or woolgrease kills attached female ticks within 24 hr.

16% of female ticks on Cheviot yearlings in spring are attached in long-woolled areas. For this and other practical reasons, sprays and smears are useless for sheep. A derris-Fuller's earth dust is as effective against ticks as the dip described above but is difficult to disperse in woolled areas. The chief drawback with derris dust, however, is that at certain times it can blind half the sheep. For sheep, the dip is superior to any known dust.

Young lambs cannot be dipped: they must be treated on the pasture by methods described. Derris-Fuller's earth dust is superior to a derris smear, giving protection of a similar order to the dip on sheep. Among thousands of lambs dusted, very much less than 1% had their eyes affected.

Cattle can be treated in stalls by washing or dusting, if used to handling; if not (with the exception of suckling Galloway and Kyloe cows), they can be jetted in stocks. Of several derris dusts, washes and jetting fluids, the best is one containing derris, soap and water. This gives protection of the same order as that with dip on sheep.

The derris materials described are the best yet produced for anti-tick treatment of stock. Cubé (*Lonchocarpus*) root of similar rotenone content to derris root gives similar results to derris in the dip for sheep, dust for lambs and wash for cattle.

Attempts have been made to reduce tick populations on grazings by serial anti-tick treatment of stock. The maximum serial interval possible with derris-containing materials is 2–3 weeks. Because lambing prevents dipping for about 6 weeks, this permits about 50% of the female ticks to escape and breed on the normal sheep farm in spring. The serial interval, i.e. residual protective duration of anti-tick materials, requires to be lengthened to 5–6 weeks to prevent this escape. The research work necessary to show whether or how the duration of residual protection could be increased is suggested.

Introduction

For generations, the sheep tick *Ixodes ricinus* L. has severely limited the productiveness of farm stock in some areas of Britain. The tick was for long, however, only vaguely suspected, and the suspicion was certainly not universal (Edgar, 1909). In 1932, it was finally proved to be the vector of Louping Ill, an encephalomyelitis of sheep (MacLeod & Gordon, 1932). This finding dates the inception of systematic research on tick control by treatment of farm stock in Britain. The sheep tick is now known to be impli-

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cated in three diseases of sheep and at least one of cattle, besides several secondary, though nevertheless serious, troubles.

At first the research on 'anti-tick' materials was entirely in the hands of the dip manufacturers, with a little academic co-operation in some of the field trials. Within the last 2 years the Agricultural Research Council has taken up the problem actively (Agric. Imp. Coun. 1944).

When the writer started work in 1939, several anti-tick dips for sheep, and smears and dusts for lambs were on the market. These were the fruits of several years' commercial research on anti-tick materials and decades of research in manufacturing technology. Though an advance in the problem, they fall below the desired efficiency. There is a considerable literature on anti-tick treatment of stock overseas but little is pertinent to the British problem. Hendrick & Moore (1937) have summarized British knowledge, revealing its scantiness. Indeed, the writer soon concluded that so far as Britain was concerned no one outside the commercial laboratories appeared to have even a working knowledge of dips in general, and still less of anti-tick dips. The manufacturers were willing to co-operate within limits dictated by their commercial interests, and to supply for experimental purposes any preparations made to formulae the writer might suggest. After several (fruitless) trials of this sort, the writer decided to devote part of his time to studying closely the commercial dips for sheep in an attempt to formulate principles whereby research could proceed from a point not too far behind the manufacturer, and part to devising new preparations and methods of treatment for lambs and especially cattle, both of which had been more or less neglected in the past.

The practical and statistical methods of field testing used have been fully described in a previous paper (Milne, 1943).

Some problems of the practical treatment of different types of farm stock

The sheep tick is confined chiefly to the hill grazings in Britain (MacLeod, 1932; Milne, 1944). These grazings are mainly stocked with sheep, and, to a much less extent, cattle. No other farm stock are important in relation to the tick in this country. The main problem in the application of anti-tick materials in practical farming is the cost of labour and material in relation to the results obtained. The typical hill farm varies from 1000 to 5000 acres or more of rough hill country, stocked at one sheep to 1–5 or 10 acres. A shepherd generally looks after about 400 sheep, so that labour is scarce, sometimes only two men on a farm. A few cattle may graze with the sheep. Except in some cases where lambing takes place in fields near the farm steading (buildings), the sheep stay on their grazings all the year round. Pens, usually built near the steading, are necessary for handling sheep. The grazings may be 1–5 miles from the pens. The labour of gathering flocks to the pens (e.g. for anti-tick treatment) is, therefore, heavy and not to be lightly or frequently undertaken.

Sheep with young lambs. Dipping, i.e. complete immersion in a bath containing insecticidal fluid, is the most efficient method of treating sheep. Unfortunately, the presence of young lambs during the tick season complicates matters. Lambs become infested within a few hours of birth, all over the body but chiefly on the legs and underparts. Very young lambs cannot be dipped for a few weeks after birth, for the following reasons: Dipping entails gathering the flock to the pens. Dams and lambs lose touch with one another in the milling confusion. The dam may not contact her starving lamb for hours afterwards. After immersion, the individual smell of each lamb, whereby the dam recognizes her own, is masked for some time. This still further delays recognition and with young ewes may result in permanent refusal of the lamb. Again, a weakly wet lamb may perish in the worst of April weather. It follows that if young lambs cannot be dipped, neither can their dams. Dipping has been done when lambs are 4–6 weeks old, yet the same difficulties in lesser degree preclude it in the eyes of most flockmasters. To meet these difficulties, and because lambs are highly susceptible to tick-borne disease, the writer tried to develop a method of hand treatment on the pastures. Parcels of 10–20 ewes with their respective lambs were 'mothered off' into a temporary pen erected on the grazing. The lambs were hand-dressed with a fairly odourless dust or smear leaving head, back and tail untreated so that the ewe could recognize the smell of her progeny. The parcel of ewes and lambs was then released into a cleared space near the pen where 'mothering up' was possible in a few minutes. The chief drawbacks are the immense labour of manipulating (gathering, mothering, etc.) the sheep, the untreated tick-infested areas of the lamb's body and the fact that no satisfactory method of hand-treating the ewes has yet been found. The field treatment of ewes with young lambs has not yet been satisfactorily solved.

Cattle. Cattle are subject to very high infestations on all areas of the body, except the back and upper sides from the top of the shoulders to the root of the tail. Ticks tend to concentrate, however, on the head, neck and between the forelegs and hindlegs—though to a less extent than in sheep. Although cattle are dipped abroad, the numbers on any tick farm or even tick district in Britain are too small to justify expenditure on swim-bath construction or the quantity of fluid required. Cattle must be hand-treated if habituated to stalls and handling, or jetted in stocks if not. In the latter case, suckling Galloway or Kyloe cows are generally too dangerous to treat in stocks. Separating them from their calves often leads to wild

excitement resulting in injury or collapse. The treatment of this class of cattle stock, common on hill grazings, remains an unsolved problem. Other classes of cattle have been successfully treated in stalls (e.g. milking cows) or in stocks (e.g. store cattle).

The foregoing outlines very briefly only the main problems of application of anti-tick materials in farming practice.

Anti-tick materials for sheep

(*a*) *Dips*

(i) *Requirements of a dip*

Many chemicals will kill ticks, but the production of a practical sheep dip is fairly complex.

The dip must be *safe*. For instance, experimental dips may cause poisoning through ingestion or skin absorption, 'scalding' of the skin, inflammation of mucous membranes, blindness, or have deleterious effects on the wool.

The dip must be *easily mixed* in water having the temperatures and compositions occurring in hill streams. Farm hands know little about making good emulsions and their time is limited.

The dip must have *lasting effect* concomitant with the practical difficulties and possible cumulative ill-effects of repeated dipping.

If possible, the dip should be *capable of dealing with all or most of the ecto-parasites present*—keds, blowflies, lice, scabmites.

The dip must be *reasonably cheap* having regard to the number of times it must be used per annum.

(ii) *The present commercial anti-tick dips*

Several experimental dips have been tested at the Aberdeen and Newcastle centres in the last few years. Discussion of these has little value since none has surpassed the general run of commercial products and none has come near the best commercial product, except, perhaps, a derris-carbolic dip (see Moore, 1943).

British commercial anti-tick dips are generally composed of sodium arsenite, cresols, and a grease, usually woolgrease but in one case thought to be whale oil. One of the chief functions of the cresols is to prevent decomposition of the grease. Only one dip contained ground derris root, until war measures cut off supplies.

MacLeod, of the Cooper Technical Bureau, who had the major share in inventing the best commercial dip to date, states (1941): 'Sodium arsenite, when applied at a concentration of 0·2 % As_2O_3, has been found to continue to kill adult female ticks attaching to sheep for a period of 10–14 days. Derris, at a concentration of 0·02 % resin, has a similar effective duration. Coal tar creosote dips confer no appreciable protection against infestation. It has further been found that the addition of woolgrease prolongs the period of effectiveness of sodium arsenite, but not of derris. Based on these findings, a protective type of dip has been evolved, containing both arsenic and derris, which protects sheep to a large extent against adult infestation for a period of two or three weeks, the protection during the first week or 10 days being of a very high order because the ticks are killed by derris before they can attach, and so the danger of disease transmission is reduced. For the latter part of the protection period the effect is due to arsenic, which kills females after they attach.' This constitutes the only published facts available about this dip. No details of tests are offered for examination.

The Chemistry Department of King's College kindly analysed the commercial dip referred to by MacLeod. Samples were taken from a pail of dip mixture purchased 12 months previously. Table 1 shows the analysis.

Table 1. *Analysis of best commercial anti-tick sheep dip*

(Nos. 4 and 5 dependable (see Table 3); the rest probably less dependable.)

Constituents	Wt. % as sold	Wt. % when diluted for use according to instructions (1 lb./5 gal. or 454 g./22·75 l.)
(1) Water	32·7	98·65
(2) Ground derris root	4·0	0·08
(3) Woolgrease:		
saponified	4·3	0·09
unsaponified	36·3	0·73
(4) Tar oils and tar acids	13·0	0·26
(5) Arsenic (as As_2O_3)	9·7	0·19
	100·0	100·00
(6) *p*H	—	8·43

Table 2 gives a typical selection of field tests (March, April or May) of commercial dips (including MacLeod's dip, Dip I) on groups of twenty sheep. Each sheep was in the bath 30 sec., the head, on which a large part of the female infestation occurs, being dipped under three or four times (see Cooper Technical Bureau, 1938). This is the minimum time for reasonable wetting of the fleece to the skin, and the maximum time practicable for the labour at a normal dipping. MacLeod (1941), in connexion with his dip, says: 'For this dip, and especially for derris dips, immersion of sheep for at least half a minute is necessary; experimental evidence, both in laboratory and field, shows that an immersion period of 15 seconds, which is a common interval in practice, results in a very marked reduction of the protection conferred.' Dips of three firms were tested. The dips other than MacLeod's (Dip I), i.e. Dips II and III, both contained arsenic, cresols and woolgrease.

Immediate effect of dip. Practically all female ticks attached at time of dipping are killed within 2 days by any of the three dips.

Residual (lasting) effect of dip. A clean sheep introduced to a tick-infested grazing during the active season picks up a varying number of female ticks every day. The infestation of the sheep would therefore go on increasing for the first 7–11 days, since no female ticks would drop off until that time had elapsed (the female requires 7–11 days for engorgement (McLeod, 1932). Thus, if a dip had immediate but no residual effect, dipped sheep would not reach the current level of infestation for their grazing until 7–11 days had passed. Hence, in the tests in Table 2, attention was directed to counts on and after the 7th–10th day after dipping.

Exps. 1 and 2 illustrate the efficiency of Dip I in a comparison of dipped and undipped (control) sheep. Exp. 2 was carried out in very favourable conditions for the dip, i.e. in a dry spell, hence there was a very marked residual effect even as late as the 22nd day after dipping. Over a wider series of tests, and with shepherds' evidence of dippings not conducted by the writer, together with a consideration of fiducial limits in counts, the value of Dip I is on the average as follows: It kills all female ticks attached at time of dipping, and has a residual effect. Practically no live female ticks are found on sheep for the first 7–10 days depending on the weather: thereafter the infestation gradually increases to equal control in 3–4 weeks after dipping, again dependent upon weather conditions (rains, etc.). These findings confirm MacLeod's statement, for which there was no recognizable published data.

Exps. 3–5 show Dip I to be superior in residual effect to Dips II and III which are expectedly similar. Since the three dips are more or less alike except that Dip I contains ground derris root in addition, derris must account for the superiority. This was confirmed by makers of Dip III who later provided an experimental dip (III*a*) identical with Dip III except that ground derris root had now been incorporated. The derris content was probably of the same order as Dip I. Exp. 6 (Table 2) shows a test of Dip I against Dip III*a* for comparison with Exp. 5.

The results shown in Table 2 do not support MacLeod's idea that 'for the latter part of the protection period the effect is due to arsenic'. If this were so, counts on the 14th–18th day would be similar, for all the dips since all contain arsenic.

At all times during the tick season some unattached unfed females may be found in the fleece. They constitute a comparatively small proportion of the female ticks on the sheep. Sometimes, especially in warm sunny weather, a fraction of these is dead, apparently having succumbed before they could attach. After *dusting* sheep with ground derris root powder (as later) the proportion of unattached unfed females in the fleece is enormously increased. Most of these in the first 2–3 weeks after treatment are either moribund or dead. The same phenomena, to a lesser extent, can be seen in sheep treated with Dip I. On sheep treated with Dips II and III, however, the unattached population, live and dead, seems to be little greater (if any) than on undipped sheep. An appreciable fraction of the female ticks picked up by sheep treated with ground derris root-containing material must, therefore, fail to attach due to stupefaction or death arising from the effects of derris. From the viewpoint of lessening tick-worry and reducing the chances of disease transmission, both arising from tick attachment, ground derris root would therefore be superior to arsenic even if they both killed the same number of ticks. Hence, Dip I is the best anti-tick dip available at present since it gives the greatest degree of residual protection. Data

TABLE 2. *Tests of commercial anti-tick dips. Av. no. of female ticks per sheep, dipped and control, at intervals of days after dipping*

(S. = statistically significant difference between means; N.S. = not significant. Owing to activity curves (see Milne, 1945) the general trend of counts throughout an experiment may be up or down.)

	Exp. 1 (Cheviot sheep)	
	10th day	17th day
Dip I	1·15	4·55
	(S)	(S)
Control (undipped)	12·95	8·80

	Exp. 2 (Herdwick sheep)		
	10th day	16th day	22nd day
Dip I	0·00	0·60	3·21
	(S)	(S)	(S)
Control	1·55	3·40	20·75

	Exp. 3 (Herdwick sheep)		
	10th day	16th day	21st day
Dip I	1·1	4·3	11·4
	(N.S.)	(S)	(N.S.)
Dip II	3·6	7·9	10·8

	Exp. 4 (Black-face sheep)	
	11th day	18th day
Dip I	2·4	10·5
	(S)	(S)
Dip II	11·1	20·1

	Exp. 5 (Black-face sheep)		
	6th day	15th day	20th day
Dip I	0·0	8·5	30·2
	(N.S.)	(S)	(S)
Dip III	0·2	20·7	54·3

	Exp. 6 (Cheviot sheep)	
	10th day	17th day
Dip I	1·60	11·85
	(N.S.)	(N.S.)
Dip III*a*	2·75	12·35

in para. (iii) and Table 3 (Exp. 2) below show that Dip I furnishes an average maximum of not more than 0·08 oz. (2·27 g.) ground derris root and 0·18 oz. (5·10 g.) arsenic (as As_2O_3) to each sheep.

Escape. During the experiments, the writer noted that some attached ticks alive at a count died within a day or two afterwards. Thus, the present method of counting does not give a complete picture of the effect of the dip, i.e. more ticks die than the counts indicate. The suggestion was made to count the female ticks nearing repletion at intervals of 7 days, the assumption being that if a tick had survived about 7 days feeding normally it must be unaffected, would remain so, and drop off to breed, i.e. escape the effects of the dip. Some ticks would, however, become replete and drop off every day. The only way to ascertain the number of ticks 'escaping' would be to devise a method of collecting all the gorged ticks dropping off each sheep as it walks the grazing over a period of 3–4 weeks. This has been found impossible so far.

Dip fluid taken from the bath. In Exp. 1, Table 3, dipping commenced with 160 gal. solution (full capacity of bath), 40 gal. were added in the course of dipping and 95 gal. were left in the bath at the end. The corresponding figures for Exp. 2 were 132·5, 107·5 and 75 gal. On emerging from the bath a sheep carries with it several gallons of dip fluid. Much of this flows back into the bath, but the sheep when finally released from the dripping pen still retains an appreciable amount. This varies with age (amount of wool) and breed (amount and type of wool). The figures for the above experiments are:

14 Apr.	Black-Face hoggs (yearlings)	0·555 gal. each
14 May.	Herdwick ewes	0·550 gal. each

Total solids. The quantity of foreign solid matter added to the bath by unclipped sheep is surprisingly high. It increased roughly in geometrical progression in Exp. 1.

TABLE 3. *Condition of bath during a normal dipping with Dip I*

(The dip manufacturers kindly undertook the estimations of tar acids, arsenic and derris; the others were done by the writer. The tar acid figures are doubtful since the method of estimation involves rather a wide experimental error.)

	Conditions of bath					
	In Exp. 1, Black-faced sheep			In Exp. 2, Herdwick sheep		
Subject	Unused	After 80 sheep	After 179 sheep	Unused	After 150 sheep	After 281 sheep
Total solids by evaporation. Dry wt. g./100 c.c.	1·22	2·51	4·91	—	—	—
Tar acids, %	—	—	—	0·27	—	0·26
As_2O_3, %	0·22	0·22	0·22	0·22	0·21	0·22
Derris resin, %	—	—	—	0·0144	—	0·0109
Surface tension, dynes/cm.	47·9	48·3	49·1	48·5	49·9	50·2

(iii) *Residual protection in relation to the number of sheep previously passed through the dipping bath and some changes in the bath*

Hobson (1939, 1941) states that by lowering the surface tension, a 'wetting agent causes dip to penetrate the fleece more readily, but it also facilitates drainage from the fleece, after immersion'. Suint, the water-soluble fraction of wool-yolk, is a powerful wetting agent. With a commercial arsenic-sulphur powder 'fly' dip, Hobson showed that the last sheep through the bath at a normal dipping are more susceptible to artificial maggot infestation than the first sheep. This result he attributes to the accumulation of suint in the bath as dipping progresses, which 'results in a smaller and less stable deposit of poison in the fleece'.

Using Dip I, preliminary observations were made on sheep and bath at different stages of a normal dipping. Table 3 shows some of the bath changes.

Tar acids (*cresols*). No reliance can be placed on the apparent reduction of tar acids (see note in Table 3), but tar acids do not confer protection (MacLeod, 1941).

Arsenic. In both experiments the arsenic content of the bath remained unchanged throughout dipping. This is interesting in view of the possibilities of differential removal of water and dissolved arsenic by adsorption on the sheep skin, wool, hair, etc., and of dilution by urine, etc.

Derris. There is a 24% decrease in derris resin content of bath at the end of Exp. 2. As much as 4 gal. excess dip fluid are said to return to the bath from each unclipped sheep as it stands in the dripping pen. Although the data are scanty, the result of Exp. 2 suggests that the drainings are robbed of an appreciable amount of derris (suspended in solution as ground-root particles) caught by straining through the meshes of the fleece. This amount will be in excess of the derris present in the ½ gal. of fluid taken away by the sheep ultimately. Probably the fleece

retains more derris in the ground-root form than it would in the liquid-extract form.

Surface tension. Estimations were made by the drop-spread method, at least 12 diameters for each dip sample. The standard was a good distilled water assumed to have a tension of 74 dynes/cm. The low-surface tension of the unused tick dip is due to wool-grease soaps and to cresols. In his experiments with an arsenic-sulphur powder dip, Hobson (1939) showed that the unused dip had a surface tension of 73 dynes/cm. which was reduced to 43·3 after dipping 264 Welsh yearlings (hoggs). The *unused* anti-tick dip (48 dynes/cm.) thus had a surface tension of a similar order as the *used* fly dip. The initial low-surface tension of the tick dip may therefore prevent the maximum possible amount of arsenic being left in the fleece (cp. Hobson, 1939). Within the limits of the present experiments, the surface tension increased slightly as dipping progressed. This increase is consistent in both experiments, is statistically significant (by the '*t*' test), but is probably negligible where the retention of arsenic is concerned. The important point is that surface tension of anti-tick Dip I, owing probably to its initial low value, is not decreased by at least 281 unclipped sheep.

TABLE 4. *Total female ticks on groups of ten sheep*

(See Table 3 and text.)

Days after dipping	Sheep numbers			Significance of differences of first and last groups (*P*)
	1–10	81–90	180–189	
		Exp. 1		
5	5	15	20	0·10
11	9	15	30	0·02
18	36	42	49	0·20
		Exp. 2		
	1–10	151–160	282–291	
10	6	22	9	0·15*
17	51	67	69	0·12

* First and second groups.

Tick counts. Table 4 shows the *total* attached female ticks on the groups of ten sheep dipped immediately after each dip sample was taken for Table 3. In only one case is the difference between first and last groups significant; but there is a consistent increase in tick totals according to the order of dipping —except on the 10th day of Exp. 2, when at least the total for the first group was less than that for either the second or third groups. Because of this consistency, groups of twenty sheep instead of ten might have shown statistically significant differences. Since surface tension does not decrease and the concentration of arsenic in the bath remains constant, such differences are not likely to be due to reduced retention of arsenic in the fleece (cp. Hobson, 1939). The progressive reduction of derris in the bath is more likely to be the chief cause.

(iv) *Grease in the dip*

The amount of grease *naturally* present in the fleece is worth consideration in relation to the amount it could acquire from the bath solution. Data are scanty. The Wool Industries Research Association (Leeds) and Dr C. O. M. Steward (Chemist, Laisterdyke Mills, Bradford) supplied some information which may be tabulated as follows:

(*a*) Woolgrease content varies in different parts of the fleece (Barker, 1931; Hobson, 1936*b*), but published figures (Barker, 1931) for medium to coarse fleeces suggest that the average grease content (ether extract) is between 8 and 18% of the 'raw' fleece weight. These figures, being based chiefly on lowland and foreign breeds of sheep, are not of much value for the present work.

TABLE 5. *Woolgrease and suint in fleeces of Hill sheep in early July*

(Figures in brackets give percentage weight in raw fleece.)

Sheep	Percentages		
	On 'raw' fleece wt. Moisture (direct loss) %	On dry wt. of wool Wool-grease %	Suint %
Hill Black-faced ewe	15·2	9·6 (8·1)	6·6 (5·6)
Hill Cheviot ewe	11·7	9·9 (8·8)	6·3 (5·5)

(*b*) Average weights of 'raw' fleeces in Scotland were: Black-face ewes and hoggs, 1941 and 1942, 3·75 lb.; Cheviot ewes, 1942, 3·90 lb.

(*c*) Dr C. O. M. Steward got two expert wool buyers to choose representative samples of wool from several thousand pounds of typical piles of wool from Hill Cheviot and Hill Black-faced ewes. Table 5 shows his estimations of moisture, woolgrease (extracted with meth. ether, sp.gr. 0·73) and suint (by subsequent extraction with 61·0*P* alcohol). These give a grease content of the fleece tending towards the lower end of the average scale of 8–18% suggested in (*a*) above.

Grease content of the fleece (and its viscosity) varies with the season. For instance, it increases from March into May as the pasture improves (and becomes less viscous in the warmer weather). It is a generous assumption that 10% of the raw fleece weight of Cheviots and Black-faces is woolgrease, and at this rate both breeds have approximately 0·4 lb. (181·4 g.) woolgrease in each fleece on the average (not including the woolgrease left on the sheep after clipping). Now in Exps. 1 and 2, Tables

3 and 4, each sheep had available to it 0·04–0·07 lb. (18·14–31·75 g.) woolgrease from the bath solution (calculated from data in (ii) and (iii) of this section), assuming each sheep got its share. Hence, the amount of grease a sheep could acquire from the bath at a normal dipping is at most only 10–17% of what it already has in its fleece. This is interesting in face of the fact that woolgrease in the dip prolongs the effect of sodium arsenite.

In sheep, the thick of the fleece is more or less unsuitable for tick attachment; in cattle, ticks can attach anywhere on the body surface. This difference may be due partly to the greasiness of the fleece as compared with the hairy coat of cattle. Possibly woolgrease globules in the bath emulsion might clog the breathing pores of the female ticks, and the following very crude tests were made on undipped Cheviot sheep. (Smearing does not mean smothering but only a light filming.)

Test 1. On one lamb, four recently attached female ticks were lightly smeared with vaseline: 24 hr. later, three out of the four ticks were dead *in situ*.

Test 2. On one ewe, eleven female ticks, several days gorged, were treated as in Test 1: 24 hr. later all eleven were dead.

Test 3. Applied very thin smear of crude woolgrease to three female ticks on one ewe and two on another at various stages of engorgement from newly attached to within a day or so of full engorgement: 24 hr. later all five were dead. These experiments do not show definitely whether the ticks died owing to a film of grease clogging the breathing pores but they suggest a line of research. In bygone days shepherds used to grease lambs with bacon fat against ticks, and in 1942 a farmer reported greater protection from ticks on dipping ewes immediately after smearing the attachment sites with bacon fat.

(v) *Anti-tick dips and other ecto-parasites*

In addition to the tick the sheep is subject to attack by keds (*Melophagus ovinus*), blowfly larvae (chiefly *Lucilia sericata*), lice (*Trichodetes ovis*) and scabmite (*Psoroptes communis* var. *ovis*). Various dips have been evolved commercially for each ecto-parasite. Two or more of the pests may be present at the same time. Costs and labour make it imperative that dipping for one pest should be, if possible, efficacious against the other pests present. Arsenic will deal with scab (see Sheep Scab Orders), derris with keds (our experiments confirmed this) and lice. Cresols are also effective against lice. The best dips for blowfly so far, however, are the arsenic-sulphur types. Hence, Dip I is effective against all parasites except blowfly larvae—provided the requisite double dippings are done in the case of lice, keds and scab. Dip I is therefore a fairly all-round dip. In some areas, however, blowflies are active at the same time as ticks (Milne, 1944). While Dip I is more or less ineffective against blowfly larvae, some farmers also claim that its greasiness attracts blowfly.

(*b*) *Sprays, smears and dusts*

(i) *Sprays and smears*

Sprays. To get over the difficulty of bath-fouling in dipping, the idea of spraying or jetting sheep has sometimes been mooted. The writer experimented with a powerful foot-pump but found it a tedious and wellnigh impossible job to get sheep wet to the skin in the fleeced areas. Moore (Hendrick & Moore, 1937) experimented with a powered spraying chamber in which, 'working at a pressure of 250 lb. per square inch, half a gallon of clean dipping fluid can be sprayed on the sheep in 20 seconds'. He temporarily abandoned this particular project chiefly because the sheep were not wetted to the skin. On a sheep standing naturally, the fleece hangs against the body like a thatch, especially in the long-woolled breeds like the Black-face. In the dipping bath the fleece floats upwards and is disturbed by water movement, hence the sheep is wet to the skin fairly easily. Even then it takes 30 sec. at least for thorough wetting with some dips.

In further work, Moore has concluded that spraying efficiency depends on jet diameter and fluid pressure, among other things. He states (1943) that 'almost complete saturation of the fleece' results from using a jet 'having an orifice of 1 mm. diameter, which at a pressure of 150 lb. covers a circle of one inch diameter 12 inches from the orifice. This jet penetrates through the thickest fleece to the skin, while finer sprays lock the wool together, forming a waterproof layer on the outside, and the whole solution runs off'. Dr L. E. Robinson, of the Cooper Technical Bureau, has sent the writer 'a set of photographs of a spray-dipping appliance which we have recently installed at our field research station in N.S.W., Australia. Such spraying outfits have attracted attention in Australian sheep pastoral circles in recent times. They are used for the control of *Melophagus ovinus* infestation and mostly on recently-shorn sheep. We have yet to be convinced of their efficiency'.

Smears. The idea of smearing for ecto-parasites on sheep is ancient. Methods are described in early volumes of the *Transactions of the Highland and Agricultural Society* (see Hendrick & Moore, 1937). In general, the wool was parted and the smear rubbed in with the fingers. The labour involved would preclude this from modern farming operations. However, since the tick, unlike the other parasites mentioned, infests the bare or hairy areas chiefly—head, legs, axillae and groins—the idea of smearing these parts has been reconsidered recently. There is at least one commercial smear for lambs, while Moore (1938) has even sprayed thinner oils on adult sheep with a pump. There are many drawbacks, chief among them being the danger of skin 'scalding'. In

hot sunny weather, some greases and oils have caused severe scalding in the writer's experience. Whether this arises from the grease or oil acting as a vehicle for introducing the poisonous ingredients into the skin tissues is not known. Smears cannot easily be introduced thoroughly into the woolly parts near the bare areas, where some ticks do attach. In short, smearing is a messy, tiresome job not to be compared to the swift efficiency of dipping, and never will be popular with farmers.

(ii) *Ticks in the long-woolled parts*

MacLeod (1932, 1939) rightly pointed out that the majority of female ticks are located in the bare or hairy parts, or short wool adjoining these parts on sheep. Actually, the proportion of ticks is much higher in the long-woolled parts than is thought. In wool 3–4 in. long on the breast, 5–7 ticks are often found in a row at the bottom of a single 'natural' parting of the wool. The long wool on the lower neck, breast and shoulders of individual Cheviot hoggs was accordingly compared with the bare or hairy parts (head, ears, upper neck, axillae and forelegs). From column III of Table 6, it may be calculated that in the prescribed long-woolled areas of forty-four Cheviot hoggs in April the mean percentage of the total ticks present was $16{\cdot}18\% \pm 1{\cdot}48$ with range 0–38% and standard deviation 9·84%. Further, the number of ticks in the long-woolled parts probably varies with the time of year and the breed of sheep. The falling percentage (column IV, Table 6) in the last 2 weeks suggests that the increasing greasiness of the wool as the season progresses renders the long-woolled areas uncomfortable to ticks. Qualitative observations suggest that the percentage of ticks among long wool of Herdwick sheep is even higher than that of Cheviots. It is concluded that treating bare or hairy parts alone, therefore, allows a not inconsiderable proportion of female ticks to escape and in itself would rule out smearing, since thorough wool treatment is very difficult.

(iii) *Dusts*

The idea of dusting with a dry powder seemed feasible, since powder is more easily dispersed in the fleece than a smear. Following success with a derris powder on lambs, Lyle Stewart (Stewart & Ponsford, 1939) experimented with Black-faced ewes. His sheep groups for tick counting were too small (from statistical considerations, Milne, 1943), but the consistency of his results seems to indicate that dry derris powder is slightly superior to Dip I. This he attributes to the larger concentration of derris applied to sheep in the case of the powder. The original powder (now a commercial product) was roughly 26% derris root with 74% Fuller's earth as filler, both finely ground (analysis by Chemistry Department, King's College). The writer tried to improve the powder by eight variations of the filler, keeping in mind the little-known qualities of powders, namely, 'walking' and 'sticking'. At the time, the writer was unaware of the 'toxic' effects of inert dusts, but three at least of the fillers must have been innocuous, since 'soft substances like talc, China

TABLE 6. *Numbers of attached female ticks in long-woolled and in bare or hairy areas of individual Cheviot yearlings (hoggs)*

(Long-woolled areas = long wool on lower neck, breast and shoulders; bare or hairy areas = head, ears, upper neck, axillae and forelegs. Column I, ticks in long-woolled areas; column II, total ticks in long-woolled and bare or hairy areas; column III, percentage of ticks in long-woolled areas; column IV, as in column III, but here average per cent per week per sheep.)

	Column			
Date	I	II	III	IV
4 Apr.	12	37	32	20
	4	38	11	
	9	36	25	
	8	56	14	
11 Apr.	1	54	2	20
	13	34	38	
	5	61	8	
	15	98	15	
	7	58	12	
	7	50	14	
	22	121	18	
	52	159	33	
	22	74	30	
	14	125	11	
18 Apr.	8	50	16	26
	10	59	17	
	19	59	32	
	9	56	16	
	7	40	17	
	21	73	29	
	40	142	28	
	10	68	15	
	9	89	10	
	34	102	33	
25 Apr.	5	29	17	12
	11	52	21	
	5	75	6	
	6	74	8	
	0	79	0	
	7	27	26	
	14	86	16	
	2	73	3	
	18	92	20	
	10	72	14	
2 May	4	177	2	9
	8	26	31	
	1	44	2	
	8	44	18	
	8	85	9	
	4	45	9	
	8	62	13	
	7	69	10	
	4	54	7	
	2	45	4	

the sheep may determine whether derris will cause blindness.

(v) *Dip versus dust*

A grave disadvantage in dusting ewes and hoggs with any sort of powder is the difficulty of treating the woolled parts which harbour ticks. Parting the wool by hand is impossible under ordinary farming conditions. Powder applied to the wool surface and rubbed in with the fingers does not penetrate nearer than 2 in. from the skin, and after a week there is no sign of its having 'walked' farther inwards. Although 'nests' of 6–9 unattached unfed female ticks may be found dead in the powdered areas of the fleece, living attached ticks may also be found immediately below this where the powder has not penetrated. At the end of the fourth count in Table 7, counts similar to those in Table 6 were made on six derris-powdered sheep. A total of 295 live attached ticks was found, 162 of which, i.e. 54%, were in the long-woolled parts. Thorough dispersion of powder in the fleece is extremely difficult and laborious. For efficiency and economy of labour, dusting cannot equal dipping unless material non-injurious to the eyes and with lasting effect compatible with labour consumption can be produced. Advantages of dip over dust in ewes and hoggs are as follows:

(1) The dip goes right to the skin all over the sheep usually in 30–60 sec., while to powder properly would take many minutes if possible at all.

(2) Dipping has a general beneficial effect partly due to rendering the sheep free from other parasites and, partly, due to the tonic effect of a cold bath. Powdering prescribed areas is directed only at ticks. Sheep always seem to thrive after a dipping.

(3) The concentration of derris in the dip has never caused any blinding.

Anti-tick materials for other stock

Different types of materials and treatments are required for different types of stock.

(i) *Lambs*

Because of practical shepherding difficulties (see above) dips cannot be used on lambs until 4–6 weeks after birth at the earliest. Even then dipping presents difficulties. Various commercial and experimental smears and dusts were tried. Although the best commercial derris-containing smear was equal to derris-Fuller's earth dust in tick protection, the dust is preferable because:

(1) Powdering is a cleaner job for the shepherd; his smeary hands, difficult to cleanse on a hill-side, are a drawback in lambing and foul the inoculating syringe where used.

(2) Smears tend to scald on a hot sunny day.

(3) The greased lamb is sometimes forsaken by its dam. There is no danger of mis-mothering with powder.

Lambs become tick-infested a few hours after birth. At that time the lambs are easily caught individually on the pasture by a skilled shepherd. Dusting is therefore carried out as soon after birth as possible, i.e. soon after the lamb has been licked by the ewe and has had its first suck. The whole of the lamb except the back and face is sprinkled, the powder rubbed in with the fingers, and the lamb set free head-to-wind towards the mother with a final pat to shake out excess powder. The writer has dusted and supervised the dusting of thousands of lambs, and only a very small fraction of 1% ever became affected even slightly in one eye. Provided powder is not shaken in the eye, it appears to have little or no blinding effect on lambs. The writer has several times seen a lamb surrounded by a cloud of dust immediately after release, with the mother sniffing at it—yet neither subsequently affected. No poisoning has ever resulted through liberal coating of the umbilical cord with derris powder a few hours after birth. Observation shows all attached female ticks killed within 2 days, the lamb being practically free for 7–10 days after treatment and not fully reinfested for about 3 weeks.

At dustings subsequent to the birth dusting, the lambs are collected as described before. The operator then requires a cotton-wool mask over mouth and nose. Dusting is a disagreeable job: a derris-water paste or suspension as recommended for cattle (see below) is just as effective and is pleasant to work with. Dipping with Dip I was also tried, but derris washing or dusting was found to be best. The treatment of lambs is time and labour consuming and renders treatment of dams the same. Most hill farmers would rather suffer tick troubles than embark on a *serial* lamb-treatment campaign. They generally draw the line at one or two treatments (3–4 week interval) per season. Nevertheless, treatment pays as lambs are peculiarly susceptible to tick-borne disease.

(ii) *Cattle*

Cattle were (1) dusted or washed by hand while tied in stalls; (2) similarly treated after being roped and thrown to the ground; and (3) jetted in stocks; with a variety of materials. Method (2) is not recommended, the chief objection being that in the struggle anti-tick materials may get in the eyes: in one experiment involving ninety Galloway cattle, two were permanently blinded in both eyes with derris. As for method (3), suckling Galloway or Kyloe cows, if unused to stall or handling, are best left untreated (see p. 129). In practical farming all types of cattle, if stall-tied and reasonably well-used to handling, can be treated by hand; and, if types unused to handling, all, with the exception of suckling cows, can be jetted in stocks.

In 1940, eight experiments were done involving 129 cows, heifers and calves. In six the cattle were tied in stalls and sprinkled with derris-Fuller's earth

powder which was rubbed in by hand. In the remaining two, the cattle were sprayed with a solution containing derris extract supplied by Dr W. Moore, Aberdeen. Because of the practical difficulties with these rather wild cattle, no tick counts were attempted, but examination showed that in all cases no live female ticks were present 2 days after treatment, the cattle were free of live ticks for 7–10 days, while reinfestation was not complete for about 3 weeks—a similar result as in lambs, indeed, the usual picture with derris preparations on stock. No blindness occurred in these experiments, but due care was taken to guard the eyes since a neighbouring farmer reported blindness in a heifer due to an accident with derris warble fly dressing.

The dusting was a most disagreeable job, eyes and noses of the operators being badly affected when the cowsheds became filled with a cloud of derris. A cotton-wool mask improved matters but gave rise to another kind of discomfort—sweat and partial suffocation due to the exertion required in handling cattle. A thin paste of derris-Fuller's earth powder with water, applied with a piece of sacking, was then tried on a further forty cattle. This was equally effective and, being dustless, the discomfort was completely eliminated. On this farm, three treatments at 21-day intervals carried the cattle through the spring tick season in a relatively very tick-free condition.

In 1941 the last modification was repeated. In May three lots of cattle, comprising thirty cows, fifty-six younger cattle and one bull, were washed down with the following:

Wash I. A water suspension of 2 lb. ground-derris-Fuller's earth powder and 6 pints water.

Wash II. An emulsion of derris extract in water having the same derris extract content as that of Wash I.

Wash III. A similar emulsion having one-tenth the derris extract content of Wash II.

The results were similar to those of the previous year with dry derris powder and with derris powder water paste in all three cases, except that Wash III showed rather less protection after 14 days than Washes I and II. Stall-tied cattle were washed at the rate of 3–5 min. each.

In 1942, ninety cattle were washed with Wash I of 1941 and a new one, Wash IV, a water suspension of ½ lb. ground derris root *without filler* and 4 oz. soap in 8 pints of water. Wash I contained 1¾ oz. resin (derris extract)/gal., and Wash IV 1½ oz. resin/gal., the latter being equivalent to the Warble-fly Dressing laid down by the Ministry Order. The previous 2 years' findings on tick protection with derris-containing materials were confirmed again. With the inclusion of soap, however, over 50% less wash was required owing to increased wetting power, and the application was much easier and therefore quicker. With a soap wash 1–2 pints only are necessary for one cow: 2 oz. of soap, instead of 4, would have been sufficient. In this experiment the backs were also washed with complete success against warbles.

The reduction of a tick population on a grazing by anti-tick treatment of stock

Anti-tick treatment of stock has two partially separable aspects: the protection of stock from tick 'bite' (with its consequences) and the reduction of tick population on a grazing. Ideally anti-tick treatment should embrace both aspects to the maximum. In practice, the stock protection aspect suffers in the face of the population reduction aspect. For instance, with arsenic, ticks are attached before they die, i.e. the stock must suffer tick bite in order that ticks may be killed. With derris-containing materials, this drawback is lessened, since an appreciable number of ticks are prevented from attaching by previous killing or stupefaction (see before).

In the hill areas of the four northern counties of England there is one sheep to 1·85 acres and one 'beast' (all kinds of cattle excluding calves) to twenty-five sheep (Wheldon, 1941). The writer is completing observations on the relative host potentials of sheep as opposed to the total wild mammal and bird fauna. At the moment, it seems safe to say that sheep and cattle have a very much higher host potential for *female ticks* than all the rest of the wild fauna put together. The data, however, require fuller study. Most people have held the general view that sheep, and to a lesser extent cattle, are the mainstay of the existing tick populations on hill grazings (the most suitable habitat, Milne, 1944). Hence, it has been argued, the killing, or otherwise preventing from feeding, of all ticks coming on sheep and cattle would ultimately reduce a tick population considerably. This means serial treatment of stock during the tick seasons.

Serial anti-tick treatment of stock is directed mainly at the female tick. The idea is to break the life cycle at the reproductive stage. The female requires a blood meal before it can lay eggs, i.e. it must spend 7–11 days on the host. The aim is to prevent any replete females falling back on the ground to breed. To achieve this in the case of sheep, dipping at weekly intervals would be necessary if the dip did no more than kill ticks attached at the time of dipping (e.g. cresols). Where the dip has some lasting effect after dipping (e.g. Dip I, etc.), the dipping interval can safely be extended 6–7 days beyond the time when ticks begin to reinfest the sheep. This ensures that no female ticks can complete feeding and drop off before the next dipping (with nymphs and larvae the times would be 3 and 4 days respectively). With Dip I, and indeed all the derris-containing materials for sheep, lambs and cattle, all attached ticks are killed at the time of dipping and practically no ticks attach for 7–10 days afterwards. Hence, for com-

plete efficiency against females in serial treatment, this dip would require to be used at 14-day intervals (7+7 days). A reasonable efficiency (see Table 2) would be expected even at 21-day intervals (10+11 days). Table 9 shows what can be expected from treatment with Dip I in a breeding flock. In the spring tick season two dippings only can be done: the first within the 10 days immediately preceding 15 Apr. (commencement of lambing) and the second 4–6 weeks after 15 Apr. If the engorgement period is assumed to be 7 days, it is perhaps erring slightly on the generous side to consider the ticks of three successive weeks after dipping killed or prevented from feeding. Nevertheless, on two typical heavily infested farms in 1940 (see Table 9) 43 and 58% of the female ticks feeding on the sheep in spring would escape. With a breeding potential of 2000 eggs per female, speedy and spectacular results cannot be expected.

TABLE 9. *Average per sheep of weekly female tick counts on ten sheep in* 1940 *on two farms showing times* (†) *of possible dippings and the ticks accounted for* (*) *by these two dippings*

	Farm A		Farm B	
Period	Date	Av. no. female ticks per sheep	Date	Av. no. female ticks per sheep
I	18 Mar.	2·7	21 Mar.	7·1
	25	10·6	28	41·4
	1 Apr.	18·2	4 Apr.	33·2
II	†8	41·6*	†11	83·4*
	15	48·0*	18	73·8*
	22	33·9*	25	65·9*
	29	90·1	2 May	65·1
	6 May	103·7	9	18·4
	13	53·6	16	11·0
III	†20	35·7*	†23	5·7*
	27	24·2*	30	2·7*
	3 June	38·3*	6 June	1·1*
	10	10·1		
	17	8·6		
	24	6·2		
	1 July	3·9		
	8	2·8		
	15	1·7		

Table 10 compares the infestation levels for 5 years on ten sheep on a farm (A) where no serial dipping was carried out and a farm (B) where the two possible spring dips (see above) had been done from 1937 to 1942. Comparing the greatest and least values in each of the column (ii)'s the probability (*P*) is less than 0·01 for *both* farms, i.e. the data show no statistically significant effect of the dipping on farm (B). On the other hand, counts on groups of twenty sheep might have given more satisfactory data in this experiment (cf. Milne, 1943).

Where the stock is 'eild', i.e. non-breeding, or where the tick activity season occurs outside lambing time (e.g. north-east Scotland, Hendrick, Moore & Morison, 1938; Moore, 1938, 1939; Milne, 1944), dipping at 2–3 week intervals is possible. Moore (1938, 1939, 1943) gives graphs (of tick counts on six control sheep, rather a small group for comparing annual levels—see Milne, 1943) claiming progressive tick population reduction, consistent over 6 years from serial dipping of an 'eild' flock in north-east Scotland. This was latterly accomplished by 'an ordinary carbolic dip with 2 lb. derris powder (containing 5% rotenone) per 100 gallons... the most successful yet tried' (Moore, 1943). The following factors must be taken into account when considering Moore's work: (*a*) our present method of assessment by tick counting does not quite show the full lethal effect of dips (see 'escape', p. 132); (*b*) Moore may be working with a different physiological race of ticks (see Milne, 1945); (*c*) dip effectiveness seems to vary with the season (no exact data) and the bulk of Moore's dipping was done in summer while the writer's was done in spring. Allowing for all these, however, it is difficult to accept his second sentence: 'In practice, for a tick eradication experiment, we have found it is only necessary to dip sheep every 5 weeks with the above (derris-carbolic) dip. While a few ticks become attached between dippings, they do not appear to develop normally, and few, if any, reach the fully engorged state at the time of the next dipping' (Moore, 1943, pp. 20–1).

TABLE 10. *Average female tick infestations per week in groups of ten sheep* (i) *for the week of peak spring activity and* (ii) *for the* 5 *weeks centred on peak activity* (*see Milne,* 1944) *on farm A where no serial dipping was done and farm B where the two possible spring dips* (*see text*) *had been done from* 1937 *to* 1942

	Farm A		Farm B	
Year	(i)	(ii)	(i)	(ii)
1940	103·7	61·2	83·4	59·5
1941	85·7	52·0	25·9	16·8
1942	84·3	70·0	50·3	29·9
1943	69·5	47·2	51·6	40 8
1944	75·9*	36·2*	18·6	12·9

* Twenty sheep used instead of ten.

Unfortunately, nearly all hill farms carry breeding flocks, and in the majority of cases the height of the tick season coincides with lambing time. For complete efficiency a dip which would carry the ewes over Period II (Table 9), i.e. 6 weeks, is necessary. The present best dip, Dip I, has a serial interval of at most 3 weeks.

Certain types of cattle can easily be treated at 14–21-day intervals with reasonable efficiency. They constitute excellent female-tick traps, being individually very highly infested.

From the practical farming standpoint the difficulties of serial treatment are (*a*) cost, (*b*) labour and (*c*) the possibility of interruption of the programme owing to special seasonal work and inclement weather. Nevertheless, there are possibilities in serial treatment provided (*a*) sheep and cattle really are to a large degree the mainstay of the tick populations, (*b*) leakages caused by the too short residual effect of present tick materials can be got over, and (*c*) practical difficulties in treating certain classes of stock are solved. At present the serial treatments available are *in general* of small practical value in the problem of tick population reduction as a whole. As an adjunct to other forms of attack (e.g. destruction of suitable environment by hill land improvement) their value is increased.

Discussion on the principles of anti-tick dip research

This work, though shallow in some respects, comprises an advance in published knowledge, and has also enabled the tentative formulation of principles for anti-tick dip research. Some of these are applicable to research on all classes of anti-ecto-parasite materials for all farm animals—dips, sprays, washes, smears, dusts. The sheep being thought the most important host for the tick, we have concentrated here on the sheep dip.

So far the method used in manufacturing research laboratories has been to subject the pest, *in vitro*, to the pharmacopoeia. Then, governed by questions of cost and technical knowledge of dip mixing, the best killing agent or agents are compounded in a dip. The latter is then tested in the field. Conditions in the bath and in the sheep coat influence the efficiency of a dip.

Conditions in the bath. In the course of a normal dipping (e.g. 400 sheep) physical and chemical changes take place in the bath. Some of these have been indicated in the body of the paper. Hobson's (1939, 1941) work is on similar lines. Urine, faeces, skin secretions, tufts of wool, mud, vegetable debris, etc., are added to the bath by individual sheep. Temperature is raised by their body heat. *p*H and electrolyte concentration may be changed. There may be differential removal of water and dissolved or suspended substances by adsorption on the sheep coat. All these circumstances may change the quantitative composition, physical state (e.g. 'breaking of an emulsion or flocculation of a colloid'), wetting power and 'sticking' power of a dip. Little or nothing is known of these circumstances, which may have a profound influence on the amounts of different dip constituents finally retained on the sheep coat.

Conditions in the sheep coat. Little is known about this important class of factors. Humidity and, to a less extent, temperature in the fleece have been studied (Davies & Hobson, 1935; MacLeod, 1940). The chemistry of the fleece (Hobson, 1935, 1936*a*, *b*, 1939, 1941; Barker, 1931) and the time dip materials stay in the fleece (Hobson, 1937, 1939, 1940, 1941) have also been studied, but these studies only touch the fringe of the subject from special angles.

The term 'coat' is used here because the hairy or bare parts, where most ticks are attached, may be more important than the fleece in the tick problem. Ticks are, however, killed on the way to the attachment site which in some cases at least is over or through the fleece. Hence, the paths traversed by ticks after being picked up should be known.

The dip material retained by the sheep coat must: (1) give a speedy kill of ticks attached at time of dipping, and (2) kill as many as possible of the ticks subsequently picked up for as long a post-dipping period as possible.

The first requirement has been achieved. A possible improvement would be cheaper killing materials. The cost of present-day dips is certainly a factor operating against their use with adequate frequency.

The second requirement is relatively difficult. The need is to get the most stable acaricide to stay for the longest possible time on those parts of the sheep where it will affect ticks. This problem is a complex of (1) the physical and chemical properties of the acaricide, and (2) the physical, chemical and physiological properties of the sheep coat (skin, wool, hair). These two sets of factors are not separable.

If the acaricide could be kept exactly where it is wanted on the sheep coat it would be subject to the physico-chemical conditions of the sheep-coat environment such as temperature, humidity, and the chemistry of skin secretions. The duration of its effectiveness would then simply be the period necessary for complete dissipation of the acaricidal property by chemical and physical changes produced by the environment conditions. But even with the most stable acaricide, the chief problem is to get the material to stay where it is wanted.

The following are among the processes which prevent this: (*a*) the secreting activity of the skin, (*b*) the outward flow of yolk along the wool fibres, (*c*) the growth of wool—said to be 1 in./3 weeks at certain times of the year, and (*d*) the leaching action of rain, aided by disturbance due to winds, to body movements, and to external frictions against vegetation, rocks, the ground, etc. All these processes combine to keep the coat clean. The throwing out of foreign and waste materials is one of the natural functions of the sheep coat. From Hobson's work, and the present studies, acaricidal materials may well be away from the skin (i.e. attachment sites), if not

altogether out of the coat, in 3–4 weeks after dipping. If this be true, and it requires very detailed investigation to show the speed and extent of the coat reaction, the duration of the protective period may be governed chiefly by the 'cleansing' mechanisms of the sheep coat.

The writer wishes to thank the following: Captain the Hon. Claud Lambton, Mr F. J. Moralee and others for their generous permission to use their farm stock in the experiments. The Chemistry Department, King's College, Newcastle-upon-Tyne for analyses. The Cooper Technical Bureau, in particular Dr John MacLeod, for providing experimental materials. Dr C. O. M. Steward (Bradford) and the Wool Industries Research Association (Leeds) for data on grease and suint content of the fleece. The Agricultural Research Council and the Duke of Northumberland Memorial Fund for providing funds per Mr W. Lyle Stewart, M.R.C.V.S., who gave valuable advice.

Appendix I

A substitute for derris in war-time

The loss of our main derris supply centres (e.g. Malaya), and the 'freezing' of existing stocks of derris in this country, have resulted in derris being unavailable for anti-tick dips, dusts and washes during the war. Since the derris-containing materials described here are superior to all other anti-tick materials so far produced, the need for an accessible substitute is urgent. Dr J. MacLeod suggested *Lonchocarpus* (Cubé), a genus related to derris and growing wild in South America. The Cooper Technical Bureau kindly supplied dips, powders and washes whereby their standard derris-containing materials might be tested against materials with cubé of equivalent rotenone content substituted for derris. Both the derris and the cubé have been used in the ground root form. In two experiments with dips on sheep, four with dusts on lambs and two with washes on cattle, there was no significant difference between the effects of cubé and derris.

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Evaluation of materials for louse control

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(With 3 Text-figures)

A simple procedure for evaluation of the effectiveness of different louse-killing substances is described. Factors likely to influence the results are discussed, and it is shown to what extent differences in age, mode of application and interaction of carriers are able to influence the insecticidal effect. A number of substances was tested by the methods described and their potency against lice determined by comparison of the minimum time of exposure necessary to give 100% kill.

Introduction

While much has been published on the action of insecticides upon agricultural insect pests and exact methods have been worked out for the comparison and quantitative evaluation of the effect of different insecticidal substances, very little is known in this regard in connexion with human lice. There are a great number of substances used for control of lice mentioned in the literature (Buxton, 1939; Strong, 1942), but usually no indication is given of the method by which their action has been tested and consequently no comparison is possible. There is no generally accepted method for evaluation of insecticides for use against lice, and such methods as have been worked out for agricultural insect pests are not suited for the louse. We, therefore, studied first the reaction of the body louse (*Pediculus humanus corporis*) to different insecticides in order to find the best methods of quantitative evaluation and then compared a number of available insecticides by means of these methods.

Methods and materials

Lice were bred in the laboratory by feeding twice daily on human volunteers and were kept in the interim at a temperature of 30°C. and a relative humidity of 70–80%. The eggs, deposited by the lice on wool threads, were kept under the same conditions.

The effective dose of an insecticide is determined by the time of exposure and the concentration of poison. Usually, we determined the shortest time of exposure necessary to give a 100% kill at a given concentration of the insecticide. This value will be referred to later as minimal lethal time of exposure.

Different methods were used for contact and fumigant insecticides. For testing liquid contact poisons, batches of 5–10 lice were immersed in different dilutions of the poison and kept there for times ranging from 1 to 60 min. The contact was interrupted by placing the exposed lice immediately on blotting paper where the adhering poison was removed by careful blotting. For shorter contacts, the lice were put in batches of five on a flat glass plate and the poison was dropped on to them so as to enclose the whole batch. At the desired interval, the whole batch was quickly wiped off with a small strip of blotting paper on to a larger sheet and cleaned and dried as described above. In that way exact exposure times down to 1 sec. could be applied.

Solid contact poisons were spread evenly on a piece of cloth or filter paper (or put on the skin of human volunteers) and the lice allowed to crawl on these areas for different lengths of time. All lice were kept, after exposure, on small pieces of blotting paper under the same conditions as normal lice. Results were read after 24 hr. and, if necessary, again after 48 hr.

Fumigants were evaluated in hermetically closed glass containers, where the test substance evaporated from a small piece of blotting paper at some distance from the lice. Different time intervals as well as different concentrations were tried.

I. Factors influencing the results of evaluation

(1) *Susceptibility of the insect in its different stages*

All the various contact insecticides tested by us could be divided into two groups according to their effect upon the different stages of the insect. Members of the first group affected all the instars to the same degree, while those of the second group were more active against imagines than larval stages. None of the substances tested gave the reverse picture, that is, a toxicity higher for larvae than for adults. To the first group belong pyrethrum, ethyl alcohol and creosote; to the second, kerosene oil and normal and isobutanol. It is the youngest stage (i.e. first instars of 1 or 2 days old) which is the most resistant to insecticides of the second group (Table 1). As can be seen from Table 1, adult lice are killed by kerosene* after an exposure of 1 min., while it is necessary to

* Only the product of the Consolidated Refineries, Haifa, was tested. It is not known whether other brands of kerosene act in a similar manner.

prolong the exposure to 60 min. in order to kill first-stage larvae. Not all insecticides of the second group showed this difference to the same degree. With butanol, the minimal lethal time of exposure for first-stage larvae is twelve times longer than for imagines. The growing larvae lose this peculiar resistance rather quickly; the second instar is killed after 2 min. contact with kerosene, while the susceptibility of the third instar is equal to that of the adult

TABLE 1. *Effect of various substances on two different developmental stages of the body louse*

Insecticidal substances	Min. lethal time of exposure: Imago *a*	Min. lethal time of exposure: Larva of the first stage *b*	Ratio of susceptibility *a* : *b*
Ethanol 95%	15 min.	15 min.	1
Creosote undiluted	1 min.	1 min.	1
Kerosene oil undiluted	1 min.	60 min.	1 : 60
n-Butanol undiluted	30 sec.	6 min.	1 : 12
Xylol undiluted	90 sec.	3 min.	1 : 2

TABLE 2. *Influence of the diluent upon the insecticidal effect on the larva and imago*

Insecticidal substance dil. in kerosene oil	Min. lethal time of exposure: Imago *a*	Min. lethal time of exposure: Larva of the first stage *b*	Ratio of susceptibility *a* : *b*
Pyrin* 1/150	30 sec.	20 min.	1 : 40
Pyrin 1/50	30 sec.	30 sec.	1
Creosote 1/200	30 sec.	4 min.	1 : 8
Creosote 1/25	30 sec.	30 sec.	1
n-Butanol 1/25	30 sec.	4 min.	1 : 8
n-Butanol 2/25	30 sec.	2 min.	1 : 4

* A pyrethrum extract containing isobutyl undecylenamide as an activator (manufactured by Dodge and Olcott Co., New York).

louse. If kerosene is used as a carrier or diluent for more potent insecticides of the first group, i.e. pyrethrum, the effect is maintained. Only in the higher concentrations of the toxic principles does this difference of activity towards the various insect stages disappear.

If butanol is added to kerosene (both substances belonging to our second group) the resistance of first-stage larvae peculiar to kerosene is lowered in proportion to the amount of butanol present, but the difference in activity towards the two instars is never entirely abolished (Table 2).

No such difference in the susceptibility of different stages has been observed by us with any of the following fumigants: acetaldehyde, chloroform, ether, ethylene dibromide, ethylene dichloride.

Egg stages of insects are, in general, more resistant towards insecticides than their postembryonal stages. This holds true also for the louse. But here again a marked change in the susceptibility of the egg can be observed during the progress of development. The highest resistance is found in 1-day-old eggs. Thence a steady decrease occurs till the 5th or 6th day, after which the degree of susceptibility, as measured by minimal lethal times of exposure, remains constant till the hatching of the larvae (Table 3). As can be seen from Table 3, fumigants as well as

TABLE 3. *Effect of different substances on various embryonal stages of the body louse*

Insecticidal substance	Time of exposure	Results: Eggs in the first day of development	Results: Eggs in the sixth day of development
Creosote 1/25 dil. in kerosene	5 min.	Partial and delayed hatching	No hatching
n-Butanol 1/25 dil. in kerosene	5 min.	Partial and delayed hatching	No hatching
Sesame oil undil.	15 min.	Normal hatching	No hatching
Ethylene dibromide 5 mg./l.	20 min.	Normal hatching	No hatching

TABLE 4. *Effect of different substances upon the larva and egg of the body louse*

Insecticidal substance	Min. lethal time of exposure: Larva of the first stage	Min. lethal time of exposure: Eggs of 6 days' development
Kerosene undil.	60 min.	80 min.
Creosote 1/25 dil. in kerosene	30 sec.	1 min.
Butanol 1/25 dil. in kerosene	4 min.	1 min.
Diethylene glycol undil.	No effect in 60 min.	1 min.

contact poisons act in this case in a similar way. With certain substances, such as creosote and kerosene, the minimal lethal times of exposure for the late stages of embryonal development approach that for young larvae; with others, such as butanol and di-ethylene glycol, this value may be even lower for eggs than for larvae (Table 4).

(2) *Interaction of two or more insecticides*

If two different insecticides are applied simultaneously three possibilities exist. The two insecticides together may show an intensified or a diminished effect or no interaction at all. All three cases can be demonstrated using the louse as test object.

There is sometimes no clear differentiation in the literature between cases where an observed intensified effect is due to a mutual enhancement of the action of two substances (synergism) and those where merely a summation of the respective individual activities occurs.

1% pyrin* in kerosene kills first-stage larvae after an exposure of 2 min. For 1% creosote in kerosene the corresponding value is also 2 min. Kerosene containing 1% of pyrin *and* 1% of creosote kills in 1 min. This result is obviously due to an additive effect of the mixed insecticides. Creosote in kerosene is lethal for first-stage larvae in a 4% solution after an exposure of 0·5 min. The same concentration in alcohol, or in 1% aqueous sodium taurocholate, is ineffective even after an exposure of 8 min. Kerosene itself has no visible effect in so short an exposure time, the minimal lethal time of exposure being 60 min. In the same way butanol, if undiluted, kills first-stage larvae in 8 min., and if diluted with kerosene 1:12 in 2 min. In both these cases the addition of kerosene resulted in an activation of the diluted insecticide which cannot be explained by the insecticidal activity of the kerosene. It seems, therefore, justifiable to separate these cases from the foregoing and to classify this effect of interaction as synergism.

A striking case of antagonism results from the interaction of the two fumigants, ethylene dibromide and ethylene dichloride. An addition of a sublethal dose of ethylene dichloride to a minimal lethal concentration of ethylene dibromide reduces the activity of the latter so that the lice exposed to the mixture remain alive. This result was the more surprising as the substances are closely related chemically. Nevertheless, their mode of action on the louse as judged by the symptoms produced seems to be quite different, and this fact may offer an explanation for this curious interaction. Ethylene dichloride produces in the louse, even after short contact, a paralysis from which the louse recovers completely if the dose is sublethal. Ethylene dibromide acts without inhibiting the mobility of the affected insects; on the contrary, there seems to be an intensification of all movements. There is no doubt that respiration in cases of paralysis is lower than during active movement of the insect. Therefore, the amount of fumigant taken up in a certain time will be much larger in mobile than in immobilized insects. Hence, the addition of ethylene dichloride actually reduces the amount of fumigant acting on the insect.

The following experiment gives a detailed account of the interaction described: a batch of eight lice was kept for 1·5 min. in an atmosphere saturated with ethylene dichloride. This exposure is sufficient to stupefy the lice, but is not lethal for them. Immediately after this they were transferred to an atmosphere containing 4 mg./l. of ethylene dibromide which is sufficient to kill after an exposure of 8 min. A small amount of ethylene dichloride was added in order to prevent the revival of the lice during the exposure to ethylene dibromide. The time of exposure was 9 min. As a control the same amount of ethylene dibromide was applied for the same time to another batch of eight lice without treatment with ethylene dichloride. All lice in the control experiment died after 48 hr., while of those which got the additional treatment with ethylene dichloride six remained alive.

* A pyrethrum extract containing isobutyl undecylenamide as an activator (manufactured by Dodge and Olcott Co., New York).

(3) *The insecticidal effect of different compounds as a function of time of exposure and concentration*

A large number of experiments was carried out in order to determine the ratio of time of exposure to effective concentration with three different insecticides, pyrin, creosote and butanol. Striking differences were found between the behaviour of pyrin on the one hand and creosote and butanol on the other. If the dilutions of all three substances were made in kerosene and minimal exposure times plotted against the reciprocal value of the lethal concentrations, creosote and butanol gave within a certain limit of concentrations and times a straight line for the 100% kill (see Figs. 1, 2). With pyrin the action is much more complicated. Concentrations of 2 and 4% give practically the same killing time; from 2% downward the curve deflects so that the lethal times of exposure increase very rapidly with decreasing concentration (see Fig. 3). Pyrin at 2 and 4% kills in 0·5 min., at 1% in 2–3 min. and at 0·5% in 20 min. As shown in Fig. 1 the straight line expresses the behaviour of creosote and butanol only for the higher concentrations. In higher dilutions and correspondingly longer exposure times, the added toxic effect of the kerosene carrier itself might be responsible for the deviation from the straight-line relationship.

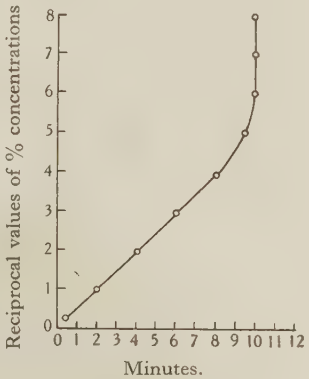

Fig. 1. Action of creosote on lice.

(4) *Effect of sublethal doses of different insecticides on lice*

In contrast to all other insecticides tested, pyrin as well as other preparations of pyrethrum have a very large zone of partial effect. At a concentration of 1:150 a certain percentage of the larvae is killed after an exposure of 0·5 min., but a 100% kill is reached only after 20 min. Concentrations from 1 to 0·2% have a similar effect. With creosote or butanol the time factor plays a much greater role; there is only a short interval between a time of exposure giving no kill and that giving 100% kill. Creosote 1:200 in kerosene is ineffective in 1 min., has a partial effect of about 20% at 2 min., of 60% at 3 min., and kills completely in 4 min. Similar observations on the relation between exposure time and percentage of effect are reported for pyrethrum by Hurst (1943).

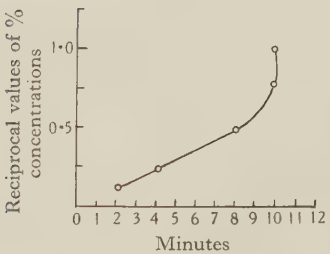

Fig. 2. Action of Butanol on lice.

(5) *The effect of mode of application on the activity of insecticides on lice*

Insecticides are in general more active if applied in liquids than in powders or ointments. This might be due to better contact in the fluid medium. It seems, however, as though the difference is not only a question of the extent of surface area which comes in contact with the poison but also of the localization of the receptors susceptible to it. In our experiments with fluid application, the whole surface of the louse is covered with the insecticide and it is not important for the results whether some parts of the body are more sensitive to the insecticides than others. By use of powders or ointments, differences in sensitivity of the surface may well be of importance. If lice are allowed to crawl on filter paper covered with a thick layer of talcum powder containing 4% of creosote, the whole surface of the louse becomes dusted and an exposure time of 8 min. is sufficient to kill. If the layer of the insecticide is thinly spread as with ointments rubbed into the skin, then only the tips of the legs and some head parts come into contact with the poison and the effect even after long exposure times may be nil. In striking contrast is the action of pyrin which remains effective even under these conditions.

II. Results of evaluation

Tables 5–8 present the results of our evaluation tests. The different insecticidal substances are arranged according to the minimal killing times. This arrangement gives a clear notion of the toxicity of the different substances with respect to the shortest possible contact time. It does not give a measure of the minimal active amount of each substance. Nevertheless, it may serve as a guide for the choice of an insecticide for lice in different situations. The final choice will, of course, not depend on the criterion of short contact

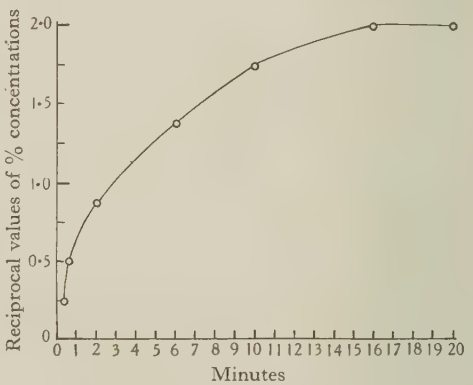

Fig. 3. Action of Pyrin on lice.

Table 5. *Liquids tested against lice by immersion method*

Insecticidal substance undil.	Min. lethal time of exposure	Symptoms
Pyrin	1 sec.	Prompt paralysis
Ethylene dibromide	30 sec.	Excitation
Creosote	1 min.	Slight paralysis
Carbon tetrachloride	1 min.	Prolonged stupefaction
Guaiacol	2 min.	Slight paralysis
Acetaldehyde 95%	2 min.	Prolonged stupefaction
Ethylene dichloride	3 min.	Prolonged stupefaction
Xylol	3 min.	Marked paralysis
Ol. caryophylli	4 min.	Slight excitation
n-Butanol	6 min.	Slight stupefaction
iso-Butanol	6 min.	Slight stupefaction
Ethanol	15 min.	Slow paralysis
Methanol	15 min.	Slow paralysis
Kerosene*	60 min.	Prompt paralysis
Ethylene glycol	>60 min.	None
Diethylene glycol	>60 min.	None

* Produced by the Consolidated Refineries Ltd., Haifa.

TABLE 6. *Solids tested against lice by crawling method*

Insecticidal substance	Min. lethal time of exposure	Symptoms
D.D.T.* undil.	2 min.	Delayed effect
Mixture of creosote 4% and pyrin 1% in talc	4 min.	Prompt paralysis
Pyrin 10% in a base of vanishing cream	10 min.	Prompt paralysis
Creosote in talc 5%	10 min.	Stupefaction
N.C.I.†	20 min.	Stupefaction
Pyrin in talc 1%	25 min.	Paralysis
Commercial pyrethrum powder	30 min.	Paralysis
p-Brom-aniline undil.	45 min.	Stupefaction
p-Dichlorbenzene undil.	1 hr. 30 min.	Delayed stupefaction
Diphenyl undil.	3 hr.	Delayed stupefaction
Diphenylamine undil.	3 hr.	Delayed stupefaction
4-Brom-diphenyl undil.	3 hr.	Delayed stupefaction
Naphthalene undil.	4 hr. 30 min.	Delayed stupefaction
4–4-Dibrom-diphenyl undil.	>24 hr.	None
Tribrom-aniline undil.	>24 hr.	None
Sulphanilamide undil.	>24 hr.	None
Thiodiphenylamine undil.	>24 hr.	None
Resorcinol undil.	>24 hr.	None
Hexyl-resorcinol undil.	>24 hr.	None
p-Chlor-xylenol undil.	>24 hr.	None
Powdered sulphur	>24 hr.	None

* Dichlor-diphenyl-trichlorethane.

† A mixture of 5% creosote, 2·5% naphthalene and 2·5% iodoform in talc.

typhus, where possibilities of a human infection depend on the length and the behaviour of the infected louse after contact with the poison. 'D.D.T.', for instance, is a very useful and powerful insecticide against lice for the prevention of lousiness. When undiluted its exposure time is only 2 min. But the affected lice die only after 16–24 hr. This means that this material cannot afford sufficient protection to an individual exposed to infected lice, because the lice may feed before they die. As D.D.T. has no marked repellent action, lice will not be prevented from lodging on a human being 'protected' by it; although they will be killed finally and will have no possibility of establishing themselves, they will have plenty of time to transmit the virus. In this respect the pyrethrines are superior to D.D.T. since they cause a final immobilization of the louse after 4 min., a time also sufficient to kill.

There is as yet no universal insecticide for lice which is ideal under all conditions and for all purposes. Measures intended to prevent lousiness will need other materials than those suited for eradicating lice from a community already infested. In the latter case ethylene dibromide used as a fumigant was found convenient and practical. As can be seen from Table 7, ethylene dibromide is a very potent substance which kills in a saturated atmosphere after 1 min. exposure. With an exposure of 8 min. as little as 4 mg./l. is sufficient for the same effect. Although the vapour pressure of this fluid is rather low, it is not difficult to achieve the low concentration necessary to kill lice. On the other hand, in this low concentration it is not irritating to human beings, and its toxicity is of such a low degree that it can be handled without special precaution. It also has no deleterious effect on clothing. Owing to its low

TABLE 7. *Killing time of various fumigants in a saturated atmosphere*

Insecticidal substance	Conc. in 1 l. air at 30°C. g.	Min. lethal time of exposure	Symptoms
Ethylene dibromide	0·180	1 min.	Excitation
Acetaldehyde	2	4 min.	Prompt stupefaction
Ethylene dichloride	0·500	5 min.	Prompt stupefaction
Chloroform	1·6	5 min.	Stupefaction
Bromoform	0·012	8 min.	Delayed stupefaction
Carbon tetrachloride	1·1	8 min.	Prompt stupefaction
Diethylether	2·2	10 min.	Stupefaction
Formaldehyde	1·3	15 min.	Stupefaction
p-Dichlorbenzene	0·012	2 hr. 30 min.	Delayed stupefaction
Naphthalene	0·0009	5 hr.	Delayed stupefaction

time alone. Several other factors must also be taken into consideration, as, for example, the effect on human beings and on clothing, the mode of application, stability, price, and last but not least the survival time of the louse after a lethal exposure. This last point has special importance if the insecticide is used as a preventive against infection with louse-borne vapour pressure it can be applied also in containers not hermetically closed. We were able to make a field trial with this substance in a labour camp and found it easy to treat from 200 to 300 persons per day, using no other equipment than a bottle of ethylene dibromide and two empty barrels as fumigation vessels. The number could easily have been increased

TABLE 8. *Minimal lethal time of exposure for 2% concentration of the insecticide*

Insecticidal substance	Diluent	Min. lethal time of exposure
Pyrin 1/50	Kerosene oil	30 sec.
Pyrin 1/50	Ethanol 95%	30 sec.
Creosote	Kerosene oil	1 min.
Creosote	Ethanol 95%	5 min.
Ethylene dibromide	Kerosene oil	5 min.
Ethylene dibromide	Ethanol 95%	7 min.
n-Butanol	Kerosene oil	8 min.
n-Butanol	Ethanol 95%	15 min.

if an additional number of barrels or other suitable containers had been available. The clothes were put very loosely into the barrels and the fumigant sprayed on each layer as they were put into the barrel. For an exposure of half an hour about 10 c.c. of ethylene dibromide per 200 l. of volume were used. This amount could be considerably reduced by the use of an atomizer. It is very important for the success of treatment to ensure access of the fumigant to the inner pile of clothing. This was accomplished simply by rolling the barrel slowly during the period of exposure. A serious drawback of this method is the fact that lice die only within 24 hr. after the treatment and may feed after the exposure.

A factor of special importance for the evaluation of substances listed in Table 6 is the stability of the compound. Several of the substances cited, D.D.T., diphenyl, brom-diphenyl, diphenylamine and brom-aniline, can be used for impregnation of clothing and laundry against an infestation with lice. The substances are dissolved in organic solvents such as alcohol, acetone or ether. Clothes are soaked in the solution for several minutes and dried in order to remove the solvent. In this process the concentration of the substance is important; for D.D.T. 0·05% is enough to kill, while for brom-diphenyl 2% and for diphenylamine 5% is necessary. Brom-aniline has a certain advantage over D.D.T. in that it produces a rather quick stupefaction. The insecticidal effect of such impregnated garments on lice will last for several weeks, and as these substances are insoluble in water the effect is not abolished by washing.

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Wireworm populations in relation to crop production

I. A large-scale flotation method for extracting wireworms from soil samples and results from a survey of 600 fields

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(With 4 Text-figures)

The wireworm survey in the Eastern Counties revealed many cases where the observed wireworm damage failed to correspond with the estimated field population. A possible explanation for this was the inaccuracy of counts made by picking wireworms out of the soil samples by hand. Tests showed that such methods recovered an extremely variable proportion of the wireworms in the samples and, on the average, only two-fifths of the larvae were obtained. A modified form of the washing and flotation technique used by Salt & Hollick (1944) was introduced for large-scale work and is described. By this method, ten samples of soil (4 in. diam. and 6 in. deep) bulked together are examined at a time and can be dealt with at the rate of 13 samples per man per hour with an efficiency of 95–100% in the extraction of wireworms. The populations estimated on 600 fields sampled between December 1942 and May 1943 have thrown more light on the size and composition of the wireworm population in grass and arable fields. Inspection of the crop results on fields tested by the washing process showed a much closer relationship between wireworm population and wireworm damage than had been obtained by the hand-sorting method in the previous year.

Introduction

Early in the war a survey of wireworm populations was undertaken throughout the country by the Advisory Entomologists. The main object of the survey was to estimate wireworm populations for use as a guide to the choice of crops on newly ploughed grassland. It was expected also that analysis of the survey results would provide valuable information about the relationships between wireworm populations and crop results under the various conditions which exist in farming practice.

Preliminary results in 1940 indicated that an estimate of the population based on the number of wireworms picked out by hand from a set of twenty random samples of soil of 4 in. diameter and 6 in. deep was accurate enough for advising on choice of crops. This method of picking out wireworms from samples broken up by hand was subsequently adopted in the wireworm survey by the Advisory Entomologists, and we shall refer to it as the hand-sorting method. However, the crop results in the Eastern Counties on the fields sampled in 1941 and 1942 caused us considerable concern and led us to question whether we could assess the potential wireworm danger in this way. The disturbing facts were that an unduly large number of fields, whose populations we had estimated to be low, suffered severely from wireworm attacks, while a large proportion of the fields with high populations were relatively free from damage. In 1941–2, on the 241 fields sampled in the 'low' and 'moderate' population categories and subsequently cropped with cereals, sixty-one cases were found, i.e. 25%, of poor crops or failures from wireworm. On the eighty-one 'high' and 'very high' fields (600,000/acre and over) cropped with cereals, only twenty-nine poor crops and failures were found, i.e. 35% (cf. Table 10 for more detailed figures). These results suggested that the relationship between wireworm population and crop result was not as simple as we had assumed. There was, however, an alternative explanation that required prior consideration, namely, that the population estimates obtained by picking wireworms out of the samples by hand did not provide a reliable guide to the population density and, therefore, our predictions about damage had not been fulfilled.

The limitations of the hand-sorting method

The hand-sorting method of examining soil samples was introduced to meet an extreme emergency when the campaign to plough up grassland began. Fryer (1942) pointed out that it would have been desirable that exploratory phases of the work should have been continued for another year or two and have been reinforced by definite experiment, but at the time the hand-sorting method was the only one available. The statistical analyses of early results had justified its general adoption, though by 1940 one centre had introduced a washing method (Bennett & Kearns, 1943), but no information as to the efficiency of extraction or the rate of working of this method has been put on record. It was only after 2 years' experience with the hand-sorting method that its limitations were investigated. The results obtained by the Wireworm Survey during the period 1939–42 are fully discussed in 'Wireworms and Food Production' (Advisory Entomologists' Conference, 1944).

It had been realized from the beginning of the survey that the hand-sorting method of examination could not recover the smallest wireworms. Larvae below 5 mm. in length were seldom found by this method, and it was the practice to include in the estimates only wireworms 5 mm. and over in length. This practice was justified on the assumption that the most serious wireworm damage would be caused by the larger larvae. Later, Salt & Hollick (1944) demonstrated that almost half the population of *Agriotes* in typical old pastures consists of larvae less than 5 mm. in length. Even so, there was no reason to believe that large larvae, assumed to be the main cause of wireworm failure, were missed in the examination.

The development of a precise washing and flotation method for the extraction of insects from the soil by Salt & Hollick (1944) made it possible to test the efficiency of the hand-sorting method for the first time. It was found by taking duplicate samples from ten fields in different parts of the country and examining them by the two methods that the hand-sorting method yielded on the average only 38% as many *Agriotes* larvae as Salt & Hollick found in their samples. The percentage found by the hand-sorting method in these samples varied according to the size of the larvae as follows: larvae less than 5 mm. in length, 11%; larvae from 5 to 10 mm., 43%; larvae over 10 mm., 80%. This was the first indication that the hand-sorting method failed to extract a large proportion of the wireworms above 5 mm. in length.

The limitations of the hand-sorting method were revealed most sharply when washing and flotation methods for extracting the wireworms were used to find out how many wireworms remained in the samples after they had been examined by the hand-sorting method. The first test was done with Salt & Hollick's apparatus and, subsequently, many more tests were done with an improvised form of the same method. These tests showed beyond all doubt that wireworms of all sizes were missed in the ordinary counts and that the proportion picked out by hand from the samples varied enormously in different fields. The two limits were 9·5% for the worst extraction and 89% for the best. Table 1 shows the results of forty-four such tests. On the average the proportion of the wireworms obtained in the dry counts was 41% of the larvae of all sizes, and 53% of the larvae 5 mm. and over in length.

TABLE 1. *Tests on residues from samples examined by hand-sorting method re-examined by washing and flotation method*

H.-S. = hand-sorting method; Res. = residue.

Field	Month	Soil: Drift	Soil: Texture	Population of wireworms all sizes: H.-S.	Res.	Total	% by H.-S.	Population of wireworms 5 mm. and over: H.-S.	Res.	Total	% by H.-S.
1	Sept.	Marsh silt	Heavy	300	400	700	42·8	300	400	700	42·8
2	Sept.	,,	,,	800	2600	3400	23·5	775	1150	1925	40·1
3	Nov.	,,	,,	525	675	1200	43·7	525	225	750	70·0
4	Nov.	,,	,,	750	975	1725	43·5	750	475	1225	61·2
5	Nov.	,,	,,	950	1075	2025	42·0	825	600	1425	57·7
6	Nov.	,,	,,	1175	700	1875	62·6	1075	375	1450	74·1
7	Nov.	,,	Medium	375	450	825	45·4	375	325	700	53·6
8	Sept.	,,	,,	225	850	1075	20·9	225	675	900	25·0
9	Nov.	,,	,,	1600	200	1800	88·8	1500	100	1600	93·7
10*a*	Oct.	Fen silt	Medium	175	700	875	20·0	175	500	675	25·9
10*b*	Nov.	,,	,,	325	325	650	50·0	325	250	575	56·5
10*c*	Dec.	,,	,,	250	325	575	43·4	250	125	375	66·6
11*a*	Nov.	Black Fen	Medium	1100	1325	2425	45·4	1050	550	1600	65·6
11*b*	Dec.	,,	,,	925	575	1500	61·6	850	200	1050	80·9
12	Dec.	,,	Light	350	250	600	58·3	350	200	550	63·6
13	Dec.	,,	,,	250	175	425	58·8	250	125	375	66·6
14	July	,,	,,	375	200	575	62·2	350	200	550	63·6
15	Nov.	,,	,,	825	450	1275	64·7	775	350	1125	68·8
16*a*	Oct.	Valley loam	Light	675	1500	2175	31·0	675	850	1525	44·2
16*b*	Dec.	,,	,,	900	1400	2300	39·1	875	975	1850	47·3
17	Sept.	Sand and gravel	,,	400	1000	1400	28·6	400	400	800	50·0
18	Sept.	,,	,,	150	1050	1200	12·5	150	450	600	25·0
19	Nov.	Chalk	Light	350	200	550	63·5	350	100	450	77·7
20	Nov.	Boulder clay	Heavy	700	1075	1775	39·4	675	825	1500	45·0
21	Nov.	,,	,,	675	1175	1850	36·5	650	575	1225	52·6
22	July	,,	,,	558	640	1198	46·5	532	350	882	60·3
23	Oct.	,,	Medium	850	350	1200	70·8	850	250	1100	77·3
24*a*	Aug.	,,	,,	700	2550	3250	21·5	700	1750	2450	28·5
24*b*	Oct.	,,	,,	650	1300	1950	33·3	600	1000	1600	37·5
24*c*	Nov.	,,	,,	1400	1325	2725	52·5	1375	1050	2425	56·7
24*d*	Dec.	,,	,,	175	825	1000	17·5	175	600	775	22·6
25*a*	Sept.	,,	,,	300	2850	3150	9·5	300	2550	2850	10·5
25*b*	Oct.	,,	,,	650	1300	1950	33·3	650	550	1200	54·2
25*c*	Nov.	,,	,,	550	1300	1850	29·7	550	450	1000	55·0
25*d*	Dec.	,,	,,	1050	500	1550	67·7	1000	450	1450	68·9
26*a*	Nov.	Oxford clay	Heavy	1975	1800	3775	52·3	1975	1050	3025	65·2
26*b*	Dec.	,,	,,	1775	1025	2800	63·7	1575	475	2050	76·8
27	Oct.	London clay	Heavy	1025	1100	2125	48·2	1000	850	1850	54·5
28	July	,,	Medium	425	450	875	48·5	425	450	875	48·5
29	Nov.	,,	,,	1025	1050	2075	49·4	1000	350	1350	74·1
30	Oct.	Gault	Heavy	225	300	525	42·7	225	300	525	42·7
31*a*	July	,,	,,	1425	2700	4125	34·5	1400	1225	2625	53·3
31*b*	Oct.	,,	,,	2075	2925	5000	41·5	1700	1875	3575	47·5
31*c*	Dec.	,,	,,	2200	4150	6350	34·7	2000	1775	3775	52·9

TABLE 2. *Repeated estimates on fourteen fields with hand-sorting method. Populations in thousands/acre of wireworms 5 mm. and over in length*

Field ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14
June–July	175	525	250	275	300	1225	275	650	—	—	—	500	950	—
Aug.		—	325	275	225	—	700	—	425	475	—	700	400	—
Sept.	450	300	400	—	—	800	350	—	—	—	275	—	—	300
Oct.	—	—	—	175	—	1850	700	750	—	—	—	775	—	650
Nov.	675	650	—	325	1075	—	1375	—	975	775	1525	2000	825	550
Dec.	—	925	—	250	850	2000	175	875	—	—	—	1550	—	1000

Examination of Table 1 shows that it is impossible to relate the incompleteness of the extraction by the hand-sorting method to any obvious single factor. It cannot be said that the method is consistently bad or good on any particular soil or individual field or at particular times of the year. Therefore, it would be impossible to compensate for the losses by applying factors based on soil types, condition or season of the year. Moreover, it is difficult to maintain that the problem arises through carelessness and inefficiency on the part of the individual. The samples were always divided amongst a team, and the composition of the team scarcely varied during the period of 6 months when these tests were carried out. It might have been thought that most of the wireworms were missed in the turf through failure to tear it up carefully enough. However, additional tests showed that most of the wireworms were missed not in the turf but in the soil. Clearly the problem is only partly due to the inefficiency of individuals, and it is more probable that efficiency is itself extremely variable under different conditions such as fatigue, and that this factor, for which there can be no solution or adjustment, is mainly responsible for the failure to pick out all the large larvae. Apart from this there are definite physiological limits to the number of wireworms which the human eye can detect in working through soil samples, and physical limits to the number of wireworms which can be separated from the soil by sieving and breaking it up by hand.

At the same time attempts were made to test the reliability of the hand-sorting method by sampling the same fields from time to time to ascertain to what extent the estimates were repeatable. These tests showed that it was the exception rather than the rule for the population estimate obtained from twenty 4 in. samples examined by the hand-sorting method to be confirmed by repeated samplings. It commonly occurred that a population estimate at one time fell in the 'safe' category with respect to potential wireworm damage and at another time the estimate placed the field in the 'highly dangerous' category. Of fourteen fields sampled at intervals between July and December 1942, not one remained in the same category for every sampling and only three gave reasonably consistent results. In the remaining twelve fields the estimates fluctuated wildly and in two cases a five-fold increase in population was recorded (Table 2).

Failure to obtain repeatable estimates is not necessarily due to the variable efficiency of the extraction technique. It might be due to the sampling error or it may represent a real change in the population at the depth of sampling between the different estimates. However, it should be said that fluctuations of this magnitude are far beyond the fiducial limits which Finney (1941) advanced for wireworm samples. Moreover, there is no evidence that vertical movements of the wireworms in the soil affect more than a small proportion of the total numbers present, and it is difficult to explain the fluctuating estimates in this way (Salt & Hollick, *in lit.*). At any rate the variability in the proportion of wireworms extracted by the hand-sorting method must account for a large measure of the fluctuation. The importance of sampling errors and of changes in the vertical distribution of wireworms in time can only be determined with a method which extracts a constant proportion of the wireworms from the soil.

Washing and flotation methods

Many investigators have worked on the problem of methods for extracting insects from the soil. By far the most satisfactory method developed up to 1942 was the system adopted by Salt & Hollick (1944). This involved the treatment of soil samples in three distinct stages: (1) washing through differential sieves, the finest of a gauze which retains even the smallest wireworms; (2) separating the organic matter from the soil by means of a high specific-gravity solution as in the Ladell method (1936); (3) separating the insects from the vegetable matter by collecting them on the benzene side of a benzene-water interface, a method used in certain branches of the food industry. This method offered an alternative to the hand-sorting method of counting wireworms, and it was felt that it could be adapted, with minor modifications, for advisory work.

In advisory work, speed of examination was always a primary consideration, and a rate of 3–4 samples per man per hour had been established as a suitable standard. It was necessary, therefore, to aim at an extraction process which did not materially reduce this rate of working. This could not be done with the method as used by Salt & Hollick (1944), which required 1½ hr. per man per sample. It was decided, therefore, to use a large apparatus capable of handling ten samples in bulk and to sacrifice the additional information provided by counts from the individual samples. For advisory work, only the population for the field treated as a whole was used. It was not our intention to aim at complete extraction of the small wireworms, as this was believed to be outside the scope of the wireworm survey and would place restrictions upon speed and increase the cost. The aim was to provide a method which extracted a consistently high proportion of all wireworms and gave a reliable estimate which could be used to advise the farmer. It was not expected that the accuracy of an apparatus designed for this purpose would compare with the method of Salt & Hollick (1944), which is said to extract not less than 99% of the wireworms.

Fig. 1 shows an apparatus which was constructed in the autumn of 1942 and has been in continuous use since. The diagram gives all necessary specifications and details of construction. Simplicity, durability and materials available were the factors deter-

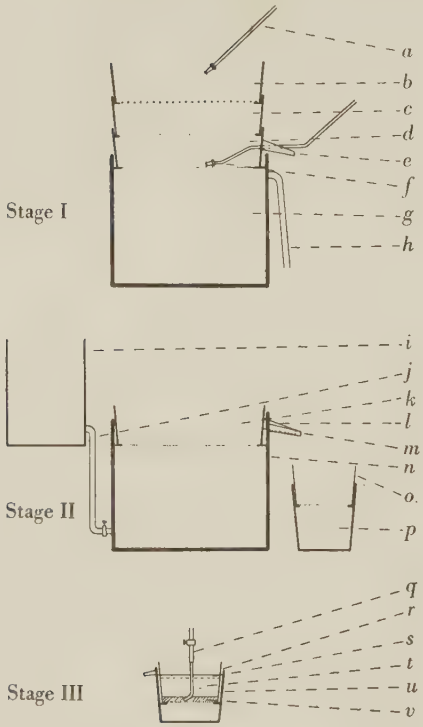

Fig. 1. Diagram of washing and flotation apparatus.

Stage I. Washing. (*a*) Water jet for washing from 1 in. main. (*b*) Coarse (2 meshes/in.) sieve. (*c*) Medium sieve (5 meshes/in.). (*d*) Fine sieve (60 meshes/in.). (*e*) Overflow lip (closed during washing). (*f*) Water jet to keep fine sieve clear. (*g*) Settling tank to collect silt. (*h*) Overflow to drain.

Dimensions and materials: settling tank galvanized 70 gal. capacity, stock size 3 ft. × 2 ft. × 2 ft.; sieves 10 in. deep to fit settling tank as shown, with walls of sheet iron galvanized or treated with rust-proof paint; strong steel gauze used for coarse sieve; monel metal gauze recommended for medium and fine sieves; lip 4 in. broad and 5 in. from top of fine sieve.

Stage II. Brine flotation. (*i*) Brine reservoir (25–50 gal.). (*j*) Brine supply pipe fitted with valve. (*k*) Fine sieve transferred from settling tank after washing. (*l*) Overflow lip on fine sieve. (*m*) Overflow lip on brine flotation tank into which (*l*) fits. (*n*) Brine flotation tank. (*o*) Collecting sieve (60 meshes/in.). (*p*) Bucket (6·10 gal. to recover brine).

Dimensions: Brine tank as settling tank except for overflow lip fitted 2 in. from top; collecting sieve 10 in. diam. and 7 in. deep with 2 in. protecting flange at bottom and walls of galvanized sheet iron.

Stage III. Paraffin flotation. (*q*) Water inlet for stirring and cleaning. (*r*) Collecting sieve transferred from Stage II after boiling. (*s*) Paraffin layer on surface of water in sieve. (*t*) Water. (*u*) Shallow bucket (capacity 4 gal.) fitted with lip as shown. (*v*) Vegetable debris at bottom of collecting sieve.

mining the form the apparatus took but there is nothing rigid about the design. For instance, circular sieves and tanks could have been used as readily as the rectangular ones adopted. It is important to set up the washing apparatus in a room with a concrete floor, and if housed within a laboratory a basement room is preferable. We have found a 5 in. drain leading into a street drain by a devious channel quite adequate to carry off the effluent which consists of water with fine silt in suspension. The washing was done with water at mains pressure of 50 lb./sq.in.

The method will be described in the five stages through which the samples pass.

(1) *Preparation of samples for washing.* The soil samples are usually placed in 4 gal. petrol tins in the field, each tin containing ten samples 4 in. diam. 6 in. deep bulked together. In the laboratory water is run into the tin until the soil is covered and the mass is allowed to soak until the soil is thoroughly broken down. The time required for this varies from 1 day for light soils to 5 days for heavy soils. At one time the samples were frozen before washing, but it was found that this did not materially improve the speed or efficiency of the extraction. The washing times required for 120 frozen and 120 unfrozen samples respectively (half the samples from each of twelve fields were frozen, and the other half soaked) were 178 min. for the frozen against 224 min. for the unfrozen samples. Thus, freezing results in a saving of only 20% in the washing time, and this is more than counterbalanced by the time taken to transport the samples to and from the freezing chamber. A more important point is whether freezing facilitates the extraction of wireworms from the soil. If this were so, it would be expected that frozen samples would yield more wireworms than unfrozen samples. This point was tested on thirty fields, ten samples from each field being frozen and the other ten soaked only. The 300 unfrozen samples yielded 1326 wireworms and the 300 frozen samples 1339 wireworms. The difference is obviously not significant and we therefore discontinued freezing as a routine practice although it is occasionally used to break down exceptionally difficult clay soils. The water jets used by us are much stronger than the jets used by Salt & Hollick, which may explain why these investigators found freezing to be essential.

(2) *Washing.* The object of the washing process is to break up the soil into a finely divided state, freeing the wireworms contained within lumps, and to separate the soil from tufted grass and large roots. The large washing sieves are set up as shown in Fig. 1 with the fine sieve (60 meshes to the inch) at the bottom. A tin of soil is emptied out into the top sieve (two meshes to the inch) and washed with water from the mains delivered through a hose fitted with an ordinary garden-hose nozzle from which the spraying attachment has been removed. Any turf in the samples must be torn into small pieces by hand

so that no wireworms can be caught up in it and prevented from passing through to the other sieves. When no more soil remains on the top sieve and the turf has been thoroughly washed, the top sieve is removed and washing continued on the middle sieve (five meshes to the inch). The holes in this gauze are 4 mm. across, allowing the largest wireworms (2–3 mm. in diameter) to pass through easily and ensuring that no lumps greater than 4 mm. in diameter remain. In practice, most of the soil is broken up into fine particles, and it is only with the heaviest soils that an appreciable number of lumps remain. Several hundred of these have been measured and the average dimensions were 3·7 mm. for the long axes and 2·5 mm. for the short axes. These lumps could contain the smallest wireworm larvae, but, as Salt & Hollick (1944) point out, it is more than likely that the presence of a larva within a lump so weakens that lump that it breaks down under the jet and the larva is freed.

The two upper sieves retain only stones and thick vegetable matter. The particles of soil and organic matter of less than 4 mm. in diameter pass through to the 60-mesh sieve at the bottom. It is not necessary to continue washing on the fine sieve, since the objects of the process have been achieved when the middle sieve no longer retains anything small enough to pass through its 4 mm. apertures.

The aperture of the 60-mesh gauze is 0·25 mm. Thus, any matter which is less than 0·25 mm. in diameter can pass through and be lost during the washing, and this would include most of the wireworms less than 4 mm. in length. It is partly to reduce to a minimum the amount of material which passes through that washing the soil on the fine sieve was avoided. We shall refer later to tests carried out to investigate the passage of material through the 60-mesh gauze.

One attachment is necessary on the fine sieve if the apparatus is to work properly. The 60-mesh gauze would be blocked very soon by soil particles, unless part of the gauze was kept free of soil. A strong jet of water directed from the centre of the sieve towards one corner is used for this purpose and is quite satisfactory, although special care is necessary with sandy soils. The gauze is soon worn away at the point where the main force of this clearing jet is directed and should be covered at that point with a thin metal plate. In time, however, the gauze becomes blocked by tiny sand grains wedged in the openings and these can best be removed by rubbing the gauze with an engineer's wire brush. This problem of keeping the sieve clear to allow the water to drain away was a limiting factor in the choice of gauze for the bottom sieve. To retain wireworms of the smallest sizes, a finer gauze would have been preferable, but the apparatus was designed for advisory work in the first instance and larger wireworms and speed were the primary considerations. For precise work on total populations, gauze with apertures of 0·15 mm. or less can be substituted.

(3) *Brine flotation.* The object of this process is to separate the soil from certain of the organic materials mixed with it. The 60-mesh sieve with contents collected during washing is transferred from the settling tank to the brine tank. This tank has a lip attached, into which the lip on the sieve fits, thus enabling floating matter to be run off into another container.

The principle is simple and well known, viz. to float the wireworm larvae and other lighter constituents to the surface of a strong salt solution, leaving the soil and other heavy matter at the bottom. Ordinary freezing salt has been found satisfactory for this purpose since it is cheap and a solution can be maintained at a specific gravity of 1·18 without difficulty. After the sieve is placed in the brine tank, the contents are thoroughly stirred so that wireworms buried in the soil float to the surface. Then more brine is run into the tank to raise the level, and when the level of the lip is reached the floating material runs off into another 60-mesh collecting sieve. This process can be facilitated by using a skimmer to push the floating material towards the lip. The procedure of stirring the contents and running in brine is repeated again and again until the surface is free of floating matter. The material in the collecting sieve consists only of matter with a density less than 1·18 and should therefore contain the wireworms.

(4) *Paraffin flotation.* It is now necessary to separate the wireworms from the large quantity of vegetable matter in the collecting sieve. This sieve is first placed in a vessel of boiling water in order to expel the air from the contents and render them heavier than water. After some time, usually about $\frac{1}{2}$ hr., the surface is practically free of floating matter, and the sieve with its contents is removed, washed very thoroughly, and placed in another vessel containing clear water. Enough paraffin to make a layer $\frac{1}{2}$ in. deep is added. Wireworms which become covered with a film of paraffin float readily to the paraffin layer and can be picked off the interface by hand. Most of the vegetable matter remains at the bottom of the sieve so that the interface is comparatively clear, and wireworms of all sizes can be seen without difficulty. Paraffin has a number of advantages over benzene, which Salt & Hollick (1944) use, viz. cheapness, comparative absence of odour and greater lifting power due to its lower specific gravity (0·81 against 0·88).

The main problem at this stage is to ensure that every wireworm comes in contact with and is thoroughly wetted by the paraffin. No completely satisfactory solution to this problem has been developed yet, and we rely on careful and thorough stirring with any simple article with a broad blade. This is supplemented by stirring with jets of water from below which throw the sunken matter up to the

surface and into contact with the paraffin. The latter system is doubly useful, as it has a cleaning action, carrying away the scum which tends to collect at the interface in samples containing large quantities of vegetable debris. If stirring is carried out in this fashion there should be very little chance of wireworms being missed. Finally, after one examiner believes that he has collected all the wireworms from a sample another worker checks the completeness of the extraction. A fresh eye usually discovers several additional wireworms. The wireworms are then removed for measuring, recording and preservation.

Performance of the apparatus

In 18 months' continuous use, the apparatus has more than fulfilled the tasks for which it was built. It has been possible to increase the number of routine wireworm tests for advisory work above the level obtained by hand-sorting methods. The peak figure obtained by hand-sorting was 158 fields in January 1942 against 187 fields by the same team using the washing apparatus in March 1943.

The rate at which the apparatus deals with samples has been established by timing all operations on forty tins (400 samples) containing soils of various kinds and turf. The average time taken by one operator to deal with one tin (ten 4 in. samples) of soils are given below:

	Min.
1. Washing loose soil through top sieve	6·0
2. Tearing up turf	2·7
3. Washing torn up turf	2·2
4. Washing soil through middle sieve	3·3
5. Washing on bottom sieve	0·7
Total for washing	14·9
6. Floating sample on brine	4·5
7. Picking wireworms off paraffin	15·5
8. Measuring wireworms	10·5
Total for all operations	45·4

Using this method samples are dealt with at the rate of 13 per man per hour. Since the different operations proceed simultaneously, it is usual to divide the work amongst a team of four who can deal with 250 samples in a day after allowing some time for routine maintenance jobs which are not accounted for in the table above. The high rate of output with this method is only made possible by the practice of washing ten samples in bulk. An identical small apparatus for washing single samples can only deal with two samples per man per hour. It is our view that in advisory work and in certain types of investigational work on wireworms, the increased output obtained by washing samples in bulk greatly outweighs in value the information sacrificed in the individual samples.

Considerable care has been taken to determine the efficiency of the method. Although the original aim was to use the method for routine advisory work for which a high degree of accuracy was not essential, it became apparent as the work proceeded that something approaching the complete population was being extracted. Two kinds of tests have been carried out: (1) *recovery tests* in which wireworms are introduced into wireworm-free soil in order to find out what proportion the method can recover; (2) *residue tests* in which the material remaining at each stage in the process is re-examined in order to see how many wireworms are lost under normal working conditions.

The recovery tests suffer from certain inherent difficulties. In the first place it is almost impossible to obtain small living wireworms in sufficient numbers to make the artificial sample comparable to a normal sample and to test the ability of the apparatus to extract wireworms of all sizes. Preserved larvae were found to be unsuitable for tests of this kind due to shrinkage and clearing and were responsible for some disturbing results in the first tests. Secondly, it is equally difficult to obtain soil that can be regarded as strictly wireworm-free and which is also suitable for washing and extraction. Wireworm-free soil from greenhouses contained large amounts of decaying organic matter which interfered with the washing process. The nearest approach to naturally wireworm-free soil occurred with soil from a rabbit warren which contained two wireworms in twenty samples. In the end we resorted to artificially prepared samples made up from the residues discarded from the washing and brine flotation stages of the process. Perhaps the best way around both these problems was found in the tests where *Tenebrio molitor* was introduced into the soil instead of larvae of *Agriotes*. But it must be admitted that it is impossible to introduce larvae into soil for tests of this kind in such a way that the normal state of affairs is reproduced. For this reason the residue tests probably give a better picture of the actual working efficiency of the method. Results of a few recovery tests are given in Table 3.

In the residue tests the material remaining after each stage of the process was re-examined by extracting with paraffin, taking small quantities at a time. There are four points in the process at which wireworms may be lost: (1) small wireworms may be washed through the 60-mesh sieve during the washing, since the hole size is larger than the diameter of the smallest wireworms in the extended condition (p. 153); (2) larvae may be left behind on the top and middle sieves, trapped in crevices or in pieces of turf; (3) they may not be floated up by the brine as a result of being buried in the soil, or in the case of small wireworms, as a result of being trapped within the larger lumps; (4) wireworms may not be floated up by the paraffin. Each of these possibilities has been investigated.

The possibility of losing small wireworms through

the 60-mesh sieve has been investigated by placing a sieve fitted with 100-mesh gauze (aperture 0·15 mm.) below the bank of sieves while samples were being washed. A quantity of silt mixed with very fine organic matter was collected in this way and examined by floating with paraffin and microscopic examination. Several tests of this kind failed to recover any small wireworms, although tests were done on samples from fields from which large numbers of small wireworms were collected on the 60-mesh gauze. The possibility of losses through collecting sieves made with 60-mesh gauze was also investigated at all stages in the process by placing a second sieve with 100-mesh gauze below and examining material collected. This, too, failed to recover any additional small wireworms. It appears, therefore, that the 60-mesh gauze used in this apparatus does not allow wireworms even of the smallest sizes to pass through.

The results of fifteen tests on the residues of the washing, brine and paraffin stages, carried out over a period of months, are given in chronological order in Table 4. It will be seen that on two occasions the number of additional wireworms found in the residues exceeded 10%. These results were disturbing and steps have been taken to prevent the recurrence of such losses by improving those parts of the method where these losses occurred. On the other hand, the average loss is only 4·5%, and of the fifteen tests twelve showed a loss of 5% or less. This is distinctly better for the extraction of all wireworms than we had hoped or planned for at the beginning.

Wireworms can be lost at each stage of the process, and the proportion lost at the different stages varies from one test to another. In one case where the loss exceeded 10%, the weakness was in the brine flotation stage, and investigation suggested that this was

TABLE 3. *Results of recovery tests with washing and flotation method*

Details of test	Wireworms of all sizes: Introduced	Recovered	Loss %	Wireworms 5 mm. and over: Introduced	Recovered	Loss %
(1) Living wireworms introduced into two tins of soil from rabbit warren. Equivalent amount of soil contained two wireworms	52	51	Est. 5	10	10	—
(2) Wireworm-free soil prepared from washing residues. Living wireworms introduced and left for 24 hr. before washing	51	51	0	—	—	—
(3) Small larvae of *Tenebrio molitor* introduced into soil prepared as in (2)	150	146	2·7	—	—	—
(4) Large larvae of *Tenebrio molitor* introduced into soil prepared as in (2)				454	450 (and 4 pieces)	1·0

TABLE 4. *Tests on residues from samples examined by washing and flotation method re-examined by paraffin flotation*

Field	Wireworms all sizes: 1st count	Residues: Washing	Brine	Paraffin	Total	Loss %	Wireworms 5 mm. and over: 1st count	Residues: Washing	Brine	Paraffin	Total	Loss %
1	112	4	0	2	6	5	75	1	0	0	1	1
2	119	0	0	6	6	5	90	0	0	0	0	0
3	76	1	12	0	13	17	61	1	9	0	10	16
4	98	0	1	0	1	1	67	0	1	0	1	1·5
5	146	0	3	3	6	4	77	0	2	2	4	5
6	79	1	0	8	9	11	45	0	0	0	0	0
7	169	4	6	2	12	7	125	3	5	2	10	8
8	147	0	2	0	2	1·5	69	0	0	0	0	0
9	129	0	0	3	3	2·5	77	0	0	2	2	3
10	175	1	5	1	7	4	108	1	4	1	6	6
11	156	0	3	0	3	1	81	0	1	0	1	1
12	225	1	6	3	10	5	180	1	2	0	3	2
13	19	0	0	1	1	5	13	0	0	1	1	7·5
14	100	0	1	0	1	1	73	0	1	0	1	1·5
15	100	0	3	0	3	3	70	0	3	0	3	4
Totals	1850	12	42	29	83	4·5	1211	7	28	8	43	3·5

due to insufficient stirring. Further tests on this point showed how important stirring is to the success of this operation. For example, in a sample yielding fifty-four wireworms, only thirty were floated up by the brine without stirring, nineteen more were brought up by stirring once, a second stirring brought up two more, a final stirring one more and when the residue was examined two more wireworms were found. Since then a closer check has been kept on the brine flotation, particularly in respect to the amount of stirring, and it is perhaps significant that no more serious losses at this stage of the process have been detected. The second case in which the loss exceeded 10% occurred at the paraffin stage and was entirely due to failure to pick off the small wireworms from the surface as the extraction of wireworms of 5 mm. and over was complete in this test (no. 6). This bears out the importance of re-examination at the paraffin stage and careful stirring to which we have already referred.

The recovery and the residue tests agree very closely in the levels of efficiency which they indicate. These tests show that the apparatus is capable of extracting an average of 95% or more of the wireworms from the samples, and that there is no need for the loss to exceed 5% provided necessary precautions are taken. Processes of this kind are not, however, automatic. Beyond 75%, the efficiency probably depends upon the care taken by individual operators to carry each stage of the process to completion.

The reproducibility of estimates of wireworm populations has been tested with the washing apparatus, repeated estimates being made on forty fields sampled at intervals between January and May 1943. The results are shown in Table 5 and should be compared with Table 2 which gave the results for repeated sampling with the hand-sorting method. It is obvious that much greater consistency in the estimates has been achieved with the washing and flotation process. The improvement is shown in Table 6, which compares the extent to which the repeated estimates depart from the means for each field with the two methods.

The estimates by the washing and flotation process were all taken in the period January–May, whereas the estimates by the hand-sorting method were taken in the period July–December. Consequently, the two series are not strictly comparable. It is possible that the numbers of wireworms found might vary more during the summer and autumn period on

TABLE 5. *Repeated estimates on forty fields with washing and flotation method. Populations in thousands/acre of wireworms 5 mm. and over in length*

Field ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Jan.	—	—	1250	1375	2725	—	—	1050	—	—	—	—	1350	1450
Feb.	—	—	1475	1800	2225	3650	1750	—	1125	1075	1600	1650	—	925
Mar.	1000	2200	1700	1375	2750	—	1700	625	1275	900	1800	1125	1675	1125
Apr.	1425	2275	1200	—	2325	2150	—	—	—	—	—	—		
May	—	—	—	—	—	—		—	—			—	—	—
Field ...	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Jan.	225	1775	1050	625	600	—	900	3775	—	—		—	—	—
Feb.	—	—	—	—	600	1350	—	3200	1100	—	625	1225	2100	550
Mar.	312	—	475	575	575	825	—	3500	—	1250	800	1175	1383	575
Apr.	—	1900	—	—	—	—	—	3375	—	925	675	—	—	—
May	—	—	—	—	—	—	625	2175	2000	—	—	—	—	—
Field ...	29	30	31	32	33	34	35	36	37	38	39	40		
Jan.	—	2200	750	2800	2050	900	1675	1575	—	—	—	—		
Feb.	4175	—	—	—	—	—	—	—	900	975	2600	2350		
Mar.	2225	—	—	—		—		—	—	—	—	—		
Apr.	—	—	—	—	—	—	—	—	1525	1975	1850	1825		
May	—	975	625	2650	1425	1050	1225	1075	—	—	—	—		

TABLE 6. *Variability of repeated estimates by hand-sorting and washing and flotation methods. Departure of the repeated estimates from the mean population of each field calculated from data in Tables 2 and 5*

Groups based on departure from the means	Percentage of 50 estimates by hand-sorting method in Table 2	Percentage of 91 estimates by washing and flotation method in Table 5
Departing from mean by	%	%
10% or more	82	60
20% or more	66	29
30% or more	54	12
40% or more	40	0
50% or more	32	0
60% or more	16	0
70% or more	8	0
80% or more	4	0

account of the effects of hatching, pupation, and possible movements of wireworms in response to changing soil conditions. In view, however, of the large numbers of wireworms missed in the hand-sorting method, we feel that the variable efficiency of the method accounts for most of the variability in the repeated sampling results in Table 2. This problem is bound up with the depth distribution of wireworms in soil at different times of the year and with the life history, which are the subjects of investigations in progress.

It is our view that this simple modification of the Salt & Hollick (1944) washing and flotation method has an application not only for routine sampling but for large-scale wireworm investigations involving the estimation of populations. So far, so much emphasis has been placed on the need for accuracy in extracting the wireworms from the soil that the enormous sampling errors have been pushed into the background. It is known that the normal statistical variability in estimating field populations is such that the customary twenty samples per field provides only a rough guide to the true population. One hundred samples or more are now believed to be necessary if the population estimate is to be regarded as close enough to the true population for moderate changes in population to be detected (Finney, *in lit.*). If these samples were to be examined individually, the labour involved would be so great that many investigations would be a practical impossibility. Moreover, the technical difficulty of obtaining samples that are strictly standard with regard to depth, diameter, etc., is also a source of inaccuracy. It is exceptionally difficult to take samples with the existing methods to an exact depth, or in which variable quantities of soil do not cling to the borer or do not fall into the hole from the sides. We feel that these errors introduce inaccuracies into wireworm counts which greatly outweigh an error of 5% in the extraction. It would be a mistake if investigations on wireworms and other soil insects were retarded by the technical difficulties associated with the extraction of the last individual from samples in which such a high degree of variability is inherent.

Size and composition of the population

During the first few months that the apparatus was in use populations were estimated on about 600 fields from various parts of the Eastern Counties. Results from these fields bear out and amplify the views expressed by Salt & Hollick (1944) regarding the size and composition of wireworm populations in grass fields.

On the grass fields sampled between December 1942 and May 1943, the average population works out at about 2,500,000 wireworms of all sizes and 1,500,000 wireworms 5 mm. and over in length per acre. The highest population we have recorded was 8,900,000/acre on an Essex marsh. Only one of the grass fields sampled had a population below 250,000/acre. It can be said, therefore, that none of the established grassland in the Eastern Counties is free from wireworms, and that the vast majority of fields contain populations that are a potential danger to susceptible crops. In Table 7 fields are grouped according to population and the number of fields in the different groups recorded. About 50% of the fields fall between the limits 1,025,000 and 2,000,000/acre, and the general picture from the table is essentially that of a normal frequency distribution.

Table 7. *Number of fields in different population groups (December 1942–May 1943). Populations in thousands/acre of wireworms 5 mm. and over in length. Fields include unploughed and recently ploughed grass fields only*

Population range	No. of fields
0– 500	32
525–1000	97
1025–1500	147
1525–2000	128
2025–2500	89
2525–3000	40
3025–3500	10
3525 and over	7

Table 8

Group	No. of fields	Average population in thousands/acre: All sizes	Average population in thousands/acre: 5 mm. and over
Unploughed	204	2551	1696
Ploughed from grass	195	2623	1749
Drilled after grass	54	1878	1312

This should be contrasted with the distribution of fields with the hand-sorting method when the great majority of the fields (two-thirds to three-quarters) fell into the categories below 600,000/acre (Table 12), and which within the categories used bears little resemblance to a normal curve.

The fields sampled between January and May 1943 have been grouped into (1) unploughed grass, (2) fields in various stages of cultivation in preparation for their first crop after grass, and (3) drilled fields sampled when the first crop after grass was in the early stages of growth; the average populations for each group have been worked out. The result is shown in Table 8.

It is clear that no significant difference exists between the figure for unploughed and recently ploughed grass fields. Apparently ploughing in these months did not lead to any sudden fall in the population. On the other hand, the average for the drilled fields is considerably lower and the figures suggest

that the later cropping operations effect a reduction of about 30% in the wireworm population. This needs to be confirmed, however, by carefully controlled tests which take into account the wireworms below the normal depth of sampling, i.e. below 6 in. on unploughed grass and 9 in. on ploughed and drilled fields.

Average populations of wireworms on fields cropped for one or more years after grass throw a certain amount of light on the rate at which the populations fall as arable cropping proceeds. Table 9 gives figures illustrating this point. It appears from the table that, on the average, a decline of more than 50% is to be expected in the numbers of both large and small wireworms during the first year of cropping. This figure has been confirmed by tests on seven fields which were sampled in December 1942 and January 1943 and again after their first crop in August and September 1943. On the seven fields taken together the population of wireworms of all sizes fell by 57% and of the larger wireworms (5 mm. and over) by 55%. On individual fields the drop recorded varied between 30 and 70% (Table 10).

Table 9 also contains figures on the effect of the second year of cropping on the wireworm population. These figures can only be regarded as rough approximations in view of the small number of fields sampled, but there is, nevertheless, indication that the rate of decline falls off after the first year of cropping. It is important to establish to what average level the wireworm population falls after several years' arable cropping. Wireworm attacks continue to be reported in predominantly arable districts and on land that has been ploughed from grass for many years, and it may be that the level at which the population finally becomes stable is higher than is usually believed or is high enough to cause damage under certain conditions.

We have been able to detect only slight relationships between wireworm population and soil type or geographical location. Soils of grass fields sampled in the course of advisory work have been classified according to the categories listed on the Ordnance Survey drift map. With the exception of chalk and black fen, for which we have a few figures only, all the categories have an average population of 2,500,000/acre, which is close to the general average for all fields (Table 8). Table 11 shows the average populations on several formations.

The position occupied by black fen in Table 11 is

Table 9. *Average populations of fields in different years of cropping after grass*

		Average population in thousands/acre to nearest 25,000		% of average population for grass fields	
Group	No. of fields	All sizes	5 mm. and over	All sizes	5 mm. and over
Grass fields	399	2575	1725	100	100
After 1st crop	29	1100	850	43	49
After 2nd crop	9	800	550	31	32

Table 10. *Wireworm populations on seven fields before and after the first crop from grass. Populations in thousands/acre*

	Population of wireworms of all sizes			Population of wireworms 5 mm. and over		
Field	Dec. 1942 and Jan. 1943	Aug. and Sept. 1943	Decline %	Dec. 1942 and Jan. 1943	Aug. and Sept. 1943	Decline %
1	2437	725	70	1363	650	52
2	637	550	12	487	150	69
3	1500	862	43	1050	512	51
4	1250	662	47	1012	662	35
5	1950	600	69	1612	412	74
6	3737	1100	71	2387	1075	55
7	1600	1225	23	1325	950	28
Average	1873	818	56	1319	630	52

Table 11. *Average wireworm populations and soils*

		Average population of wireworms	
Drift	No. of fields	All sizes	5 mm. and over
Marsh silt	55	2688	1826
Black fen	8	1216	779
Valley loam	12	2348	1531
Valley gravel	40	2239	1489
Sand and gravel	23	2094	1488
Chalk	6	1431	1096
Boulder clay	177	2386	1569
Oxford clay	84	2595	1788
London clay	13	2559	1673
Gault	7	2739	1607

worth special mention. Using the hand-sorting method for counting wireworms, we had accepted it as a commonplace that black fen soil usually yielded populations well above the average, and on wireworm survey maps it had been classified as 'very high'. It now seems likely that this impression was not correct and that the high populations recorded on black fen soils in the past reflect the unreliability of the hand-sorting method and can be attributed to the fact that it is easier to pick wireworms out of soil which breaks down readily and provides a black background.

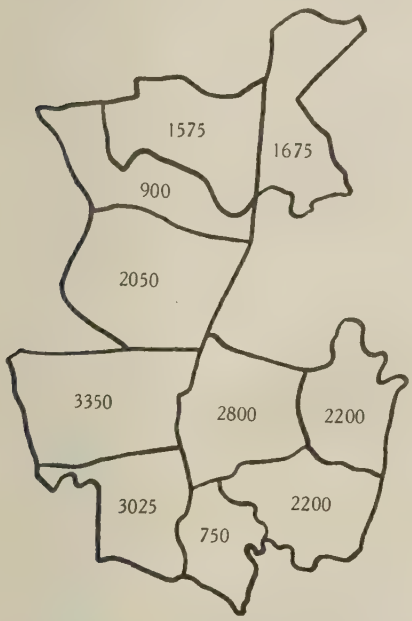

Fig. 2. Map of area sampled on Wolferton Marsh, West Norfolk, showing wireworm populations in individual fields (thousands/acre). Fields sampled in grass; all marsh grazing with similar history and uniform soil (marsh silt). Note occurrence of fields with low populations amongst fields with average and high populations.

We have not found that extensive areas with relatively uniform wireworm populations exist in the Eastern Counties. In fact, the population of any particular field seems to bear little or no relationship to the population of neighbouring fields. Fig. 2 illustrates this by showing the populations on ten adjacent fields on a Norfolk marsh which were identical in previous history and in soil type. It will be seen that the populations range from 750,000 to 3,350,000/acre and are fairly evenly distributed between these two extremes. This map is typical of the situation that has been found wherever a number of fields have been examined in a small area. We have not attempted, therefore, to survey the province and map out areas differing from each other in their average levels of population. With the possible exception of the black fen and chalk areas, referred to above, the average population in any given area lies close to the average figure for all the grass fields sampled. Our experience has led us to be very sceptical of the earlier views that there are well-defined high and low areas with respect to wireworm populations within each advisory province. Certain areas, e.g. Essex, which we had previously regarded as low areas, are now found to maintain populations of the same order as the rest of the Province. It will be interesting to see if the use of standard methods for the extraction of the wireworms from the soil in other parts of the country will level out the local differences previously reported.

The composition of the population on the grass fields sampled from January to May 1943 is shown in the histograms in Fig. 3. It shows the preponderance of small wireworms to which Salt & Hollick (1944) drew attention and the same pyramidal distribution for the wireworms taken together. In our samples the wireworms less than 5 mm. long have comprised about 40% of the total numbers, and this agrees very closely with the proportion found by Salt & Hollick (1944) on ten fields chosen at random which they sampled in conjunction with the Advisory Entomologists. The picture for the 5 months does not show much variation until April and May, when there is a marked decline in the proportion of small larvae. This would be expected from existing knowledge of the biology of *Agriotes* spp.

The composition of the population on individual fields shows great variety. In some as many as 60% of the larvae are less than 5 mm. in length and in others virtually all the larvae are longer than 5 mm. We feel, however, that an estimate based on twenty 4 in. samples cannot be expected to provide much information about the true distribution of wireworms amongst size groups of 1 mm. within a particular field. In fields sampled more than once the proportion of wireworms in the different size groups shows considerable variation from one sampling to another. Sufficient samples are needed to provide large numbers of wireworms in each size group if a reliable picture of the distribution by size is to be obtained. There is not much doubt that this variation is a statistical matter, and bound up with the theory of sampling. We hope to return to this problem of the distribution of wireworms by size in individual fields in a later paper describing results on fields that have been sampled more intensively.

The fields sampled between January and May 1943 have been grouped in order to obtain information on the composition of the population under the following conditions: (*a*) unploughed grass; (*b*) ploughed and cultivated after grass in preparation for the first crop; (*c*) first crop after grass in early stages of growth;

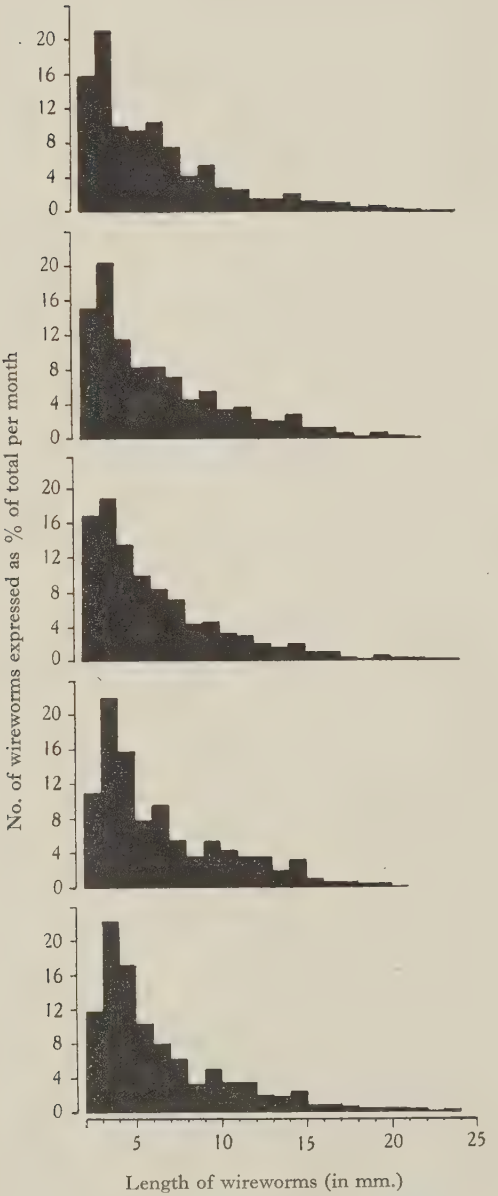

Fig. 3. Size distribution of monthly collections of wireworms from grass and newly ploughed fields. From top to bottom, Jan., Feb., Mar., Apr. and May 1943.

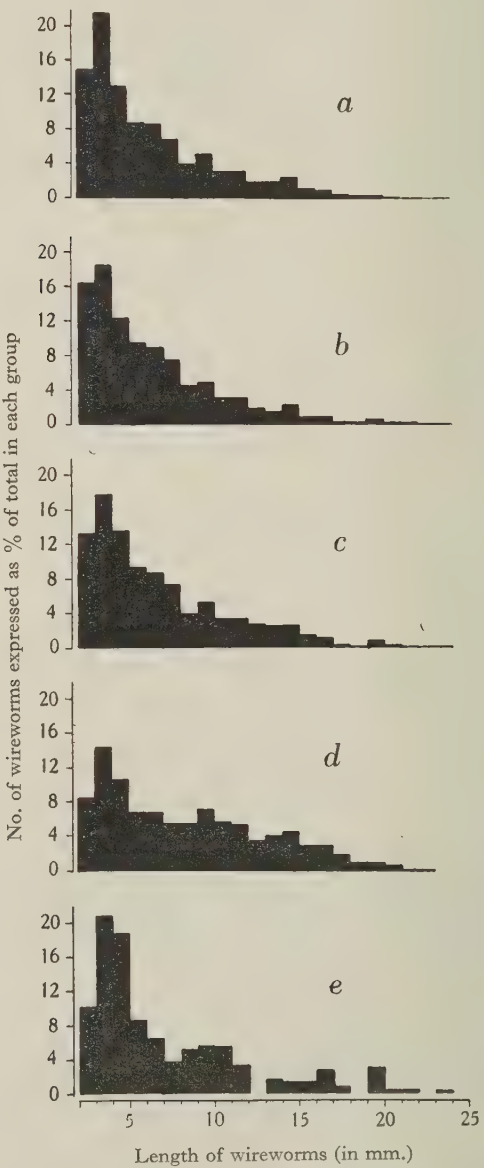

Fig. 4. Size distribution of wireworms collected from fields in different stages of cultivation. Dec. 1942–May 1943. (*a*) grass; (*b*) ploughed from grass; (*c*) in 1st crop; (*d*) after 1st crop; (*e*) after 2nd crop.

(*d*) after first crop or in early stages of second crop; (*e*) after second crop or in early stages of third crop. Fig. 4 shows in histogram form the distribution of wireworms by size in each of these groups. Although there is a decline in the proportion of small wireworms (less than 5 mm.) in the second year, this process does not appear to continue into the third-year fields. This observation needs confirmation by estimates from a larger number of third-year fields, as Fig. 4 (*e*) is based on 235 wireworms only. But it is obviously not true that the young forms automatically disappear once grassland becomes arable, and it may happen that enough young larvae emerge on arable land to maintain the population at a level that is low compared to grassland but is not negligible from the point of view of crop damage under arable conditions. These histograms should not be regarded in isolation from the earlier figures (Tables 9, 10), which showed how the absolute numbers of wireworm larvae decline in successive years of cropping.

Table 12. *Crop failures of cereals due to wireworms, 1941–2. Populations estimated by the hand-sorting method*

Category	Population	Fields sampled	Crop failures due to wireworms	Failures %
Low	0– 300,000	122	18	15
Moderate	325– 600,000	119	43	36
High	625–1,000,000	57	18	32
Very high	1025 and over	24	11	46

Wireworm populations and crop result

We have already referred to the discrepancy between the predicted and the actual damage on many fields sampled during 1941–2. In fact, our main object at the outset in replacing the hand-sorting method for counting wireworms by a washing and flotation method was to enable predictions about crop results to be made with greater confidence as a result of more accurate estimates. This hope has been realized.

Table 12 shows the number of crop failures due to wireworms that occurred in fields in the Eastern Counties in each of the four advisory categories when the hand-sorting method was in use. The table includes cereal crops only, i.e. winter wheat, spring wheat, oats and barley, and refers only to the Eastern Counties. Success or failure was estimated from the degree of wireworm damage and the number of plants established during the early periods of growth before the plants exceeded 6 in. in height. All cereal crops have been included together in the table as no difference in susceptibility to attack is apparent from the results of each crop taken singly. (Crops failing from causes other than wireworm have not been included.)

According to Table 12 the probability of a failure is three times as great on a 'very high' as on a 'low' field. Even so, a considerable number of failures, 18 out of 122, were recorded at low populations. This was a matter for some concern, since in these cases the farmers had been told that they could grow any crop without much fear of wireworm attack. At the other end of the scale, thirteen cereal crops out of twenty-four were successful in the face of wireworm populations that had led us to predict serious damage unless resistant crops were grown. But the most disturbing fact was that 176 fields, more than half the total, fell into intermediate categories for which no definite advice could be given. In the 'moderate' category advice took the form that good crops could be expected provided there was adequate consolidation and no manurial deficiency. In the 'high' category, resistant crops were recommended but susceptible crops might be successful under good growing conditions. In fact the figures in Table 12 show a negative correlation between damage and rising population in the 'moderate' and 'high' categories for which different advice had been given.

Table 13. *Crop failures of cereals due to wireworms, 1942–3. Populations estimated by the washing and flotation method*

Category	Population	Fields sampled	Crop failures due to wireworms	Failed %
Very low	0– 500	14	1	7
Low	525–1000	19	4	21
Moderate	1025–1500	51	14	27
High	1525–2000	35	14	40
Very high	2025 and over	38	26	69

The same type of analysis has been carried out for cereal crops in the season 1942–3 when the washing and flotation method was in use. The result is given in Table 13. Unfortunately, we are working with only half the number of fields in the previous table and the population categories are different owing to the higher populations of wireworms (5 mm. and over only) obtained with the new method. The five divisions were chosen quite arbitrarily for advisory work, and as no information was available as to cropping results with populations estimated in this way, the advice assumed a very flexible character and depended mainly on sorting out the very high and very low fields from the rest.

These figures show a great improvement over the figures in Table 12 from the point of view of correctly predicting the probability of damage. Thus the probability of a failure is ten times as high on a 'very high' as on a 'very low' field. To put it another way, only one farmer in fourteen lost a crop where he had been told it would be safe, and only twelve farmers out of thirty-eight grew successful crops where they had

been told to expect serious damage. Moreover, although two-thirds of the fields still occupy an intermediate position with regard to wireworm infestation, a positive correlation with rising population is evident in the three intermediate groups. Thus the probability of a failure is twice as great in the 1525–2000 group as in the 525–1000 group. This is a notable advance over the position of the intermediate fields examined by the hand-sorting method in 1941–2.

Table 14 shows the cropping results in 1942–3 for a number of crops separately. Although the number of fields for each crop is not large, the positive correlation between wireworm damage and rising population is very marked for the susceptible crops, wheat, spring wheat, oats, barley and sugar beet. Likewise the comparative absence of damage with peas, beans and especially with linseed and flax, is shown on a good number of fields. One of the most interesting features of these figures is the evidence that barley is less susceptible to wireworm attack than the other cereals, winter and spring wheat and oats. However, evidence from more fields is necessary before we can regard this point as established. The high susceptibility of oats and spring wheat shows in the figures, and this is in line with views based on crop results.

There is no doubt from these figures that the introduction of the washing and flotation process for estimating wireworm populations improved the correlation between predicted and actual damage to a

TABLE 14. *Wireworm populations and crop results with different crops, 1942–3. Populations of wireworms 5 mm. and over in thousands/acre*

Crop	Population category	Population	Fields examined	Crop failures due to wireworms	Wireworm failures %
Wheat	Very low	0– 500	3	0	0
	Low	525–1000	6	2	33
	Moderate	1025–1500	14	6	43
	High	1525–2000	6	3	50
	Very high	2025+	4	2	50
Oats	Very low	0– 500	4	1	25
	Low	525–1000	4	2	50
	Moderate	1025–1500	5	3	60
	High	1525–2000	7	3	43
	Very high	2025+	13	12	92
Barley	Very low	0– 500	7	0	0
	Low	525–1000	8	0	0
	Moderate	1025–1500	26	4	15
	High	1525–2000	20	6	30
	Very high	2025+	18	9	50
Spring wheat	Very low	0– 500	0	0	—
	Low	525–1000	1	0	0
	Moderate	1025–1500	6	1	16
	High	1525–2000	2	2	100
	Very high	2025+	3	3	100
Sugar beet	Very low	0– 500	2	0	0
	Low	525–1000	3	0	0
	Moderate	1025–1500	6	4	66
	High	1525–2000	5	5	100
	Very high	2025+	7	6	85
Beans	Very low	0– 500	4	0	0
	Low	525–1000	0	0	—
	Moderate	1025–1500	6	3	50
	High	1525–2000	4	1	25
	Very high	2025+	7	0	0
Peas	Very low	0– 500	1	0	0
	Low	525–1000	6	0	0
	Moderate	1025–1500	17	0	0
	High	1525–2000	15	1	7
	Very high	2025+	17	1	6
Flax and linseed	Very low	0– 500	1	1	100
	Low	525–1000	6	0	0
	Moderate	1025–1500	21	0	0
	High	1525–2000	17	0	0
	Very high	2025+	20	0	0

marked degree in the season 1942–3. Still it cannot be said that high infestation is invariably associated with wireworm attack and low infestation invariably with freedom from attack. Anomalous instances still occur where fields whose populations are known to be low fail completely from wireworm attack, while other fields with high populations remain undisturbed. Until these anomalous cases can be satisfactorily explained, entomologists cannot be said to understand the wireworm problem fully. It is important to investigate these anomalies and resist the tendency to dismiss them as exceptions to a general rule. Experiments designed to explain these anomalous results might throw a great deal of light on the factors which govern wireworm attack and indicate appropriate methods of control.

The experiences reported in this paper bear out the view that careful quantitative work on wireworm populations is essential to an understanding of the wireworm problem in relation to crop production. There is need for precise data on the limits of the field sampling technique under all agricultural conditions. There is also need for information on the vertical and horizontal distribution of wireworms as a part of a study of their distribution and behaviour in land ploughed from grass. Information which would provide some guidance on appropriate methods for controlling wireworm populations might be expected to emerge from investigations of this kind. We hope to report progress along these lines in subsequent papers.

The results embodied in this paper were obtained in the course of the advisory wireworm sampling carried out in the Eastern Counties. We wish, therefore, to acknowledge our indebtedness to the Advisory Entomologist, Mr F. R. Petherbridge, M.A., in whose department the work was done, and who gave us every encouragement and help in our efforts to introduce new methods. Our thanks are due also to the firm of D. Mackay, Cambridge, and their engineers, Messrs Forsdyke, who built the apparatus and who co-operated willingly at all times, often at considerable inconvenience to themselves. Finally, we must record the contribution made by our technical staff, Messrs W. G. Clark, A. M. Cornell, W. S. Darkin, P. S. B. Digby, B.A. and K. T. Schatzky, who have borne much of the laborious field work and tedious laboratory work which is still inevitable in wireworm population studies.

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Observations on the biology and experiments on control of the mangold fly (*Pegomyia betae* Curtis) on sugar beet

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(With 2 Text-figures)

The fly (*Pegomyia betae* Curtis) was studied in its ecological and phenological aspects in central Nottinghamshire, and experiments on the lines of Continental workers, using sodium fluoride and molasses spray, were carried out.

It is shown that some pupae from the second generation fail to emerge in the late summer but emerge in the spring of the following year, rather earlier than the pupae resulting from the third generation. The flies resulting from this early emergence are less fertile than those appearing later.

Methods of control discussed include cultural, biological and chemical means. Delayed drilling (not before mid-May) helps to avoid the attack of the first generation. A rapid increase in number of parasites was observed and resulted in the annihilation of a large population of the fly. Treatment with molasses/sodium fluoride bait spray gave promise of control.

Introduction

Since 1929–30 attacks by *Pegomyia betae* Curtis have been slight, but in recent years it has again become increasingly prevalent in the Sherwood Forest area of Nottinghamshire. It has been claimed that up to 40% of the seedlings of sugar beet have been lost. The great increase of the sugar-beet acreage may have tended to build up a large population of the flies.

This species has been of more importance on the Continent than in this country, and there are numerous publications dealing with it. Cameron (1914) gave an account of it in this country, and a good monograph was published by Bremer & Kaufmann (1931). A long and exhaustive series of studies by Blunck *et al.* ended with the publication of the 11th Report (1933).

It was decided to study the fly in its ecological and phenological aspects in central Nottinghamshire, and at the same time get some experience of the most recent continental control measures in this country. The farm chosen for the experiments was Cavendish Lodge, Clipstone, where Mr E. Shaw Browne had reported attacks of increasing severity over several years, the damage in some years being very considerable. A plan of the fields under observation is given in Fig. 1. Owing to the unsuitable nature of the soil, which contains large pebbles derived from the Bunter Sandstone, it was not practicable to test rolling as a means of control.

Notes on the biology of Pegomyia betae Curtis

First generation. In order to establish the dates of emergence of the first generation of *Pegomyia*, pupae were collected in the autumn of 1942 and retained in pots, out-of-doors, throughout the winter of 1942–3. Blunck *et al.* (1933) state that they emerge in the forenoon. They feed on carbohydrates on various plants and they ingest water at the same time. Adults began to emerge during the first week of April and continued until 24 Apr.: the maximum emergence was 12–13 Apr. This was rather earlier than anticipated, so on 13 Apr. further search was made in a field which had carried sugar beet in 1942 and had suffered a severe infestation. On this date thirty-three pupae were collected; fourteen of these were found to be empty (emerged) on collection. Owing to their apparent fresh nature it was considered that these fourteen empty cases were of flies that had but recently emerged. The first emergence from the other nineteen pupae occurred on 28 Apr. It appears then, that in the spring of 1943, the first generation emerged in two distinct phases, the first fortnight of April and end of April to first week of May. This may be accounted for by some pupae from the second generation failing to emerge in the late summer, but emerging in spring of the following year, rather earlier than the pupae resulting from the third generation. There is evidence that this third generation is only partial. Rambousek & Neuwirth (1931) state that flies are on the wing at the time of the flowering of sloe and cherry, and a second flight often takes place somewhat later consisting of adults that have developed from eggs laid in the preceding October if the autumn was warm.

Oviposition in the field was first noted on 3 May as soon as the first beet seedlings were showing above the soil, and on this date many flies were noted in the field, basking in the sun close to a hedge, sheltering from a stiff breeze. Oviposition was very slight until 11 May when greatly increased oviposition was observed. It would seem that the early slight oviposition was from the first brood of flies, while the main first generation began oviposition about 11 May, and continued until 28–29 May. This heavy oviposition completely masked any data that might have been

obtained giving the ending of the first early oviposition. Maximum oviposition appears to have been 20–21 May (see Fig. 2).

Second generation. Pupae from the first generation were obtained on 2 June, and the first emergence from this material was noted on 18 June. In the field, some flies must have emerged rather earlier, as oviposition of this generation was first noted on 16 June. The peak emergence in the field, however, appears to have been 24–25 June. Oviposition of this generation was completed by 10 July. By 28 July, this second generation appears to have been complete, as no larvae could be found feeding in the leaves and there appeared to be a complete pupation. This generation seems to be well defined and much more clear-cut than the first. It was noted to be parasitized, as *Opius* sp. were observed in numbers during the larval period of *Pegomyia*. *Microbracon* sp. also occurred.

Third generation. Fifty pupae from the second generation were collected on 28 July. From this material, fifteen *Pegomyia* emerged between 7 and 13 Aug. and five parasites (*Opius* sp.) emerged 16–27 Aug. Up to the end of the year these twenty were the only emergences from this material—a further indication that a proportion of the second generation pupae do not develop into the third, but overwinter and presumably are responsible for the early emergences of the first generation in the following spring. Twelve more *Pegomyia* emerged from this material 12 Apr.–3 May 1944. No more *Opius* sp. emerged, indicating that there was a full generation of the parasite the previous summer. The emergence figures corroborate the high parasitism of the second generation—in the region of 20%.

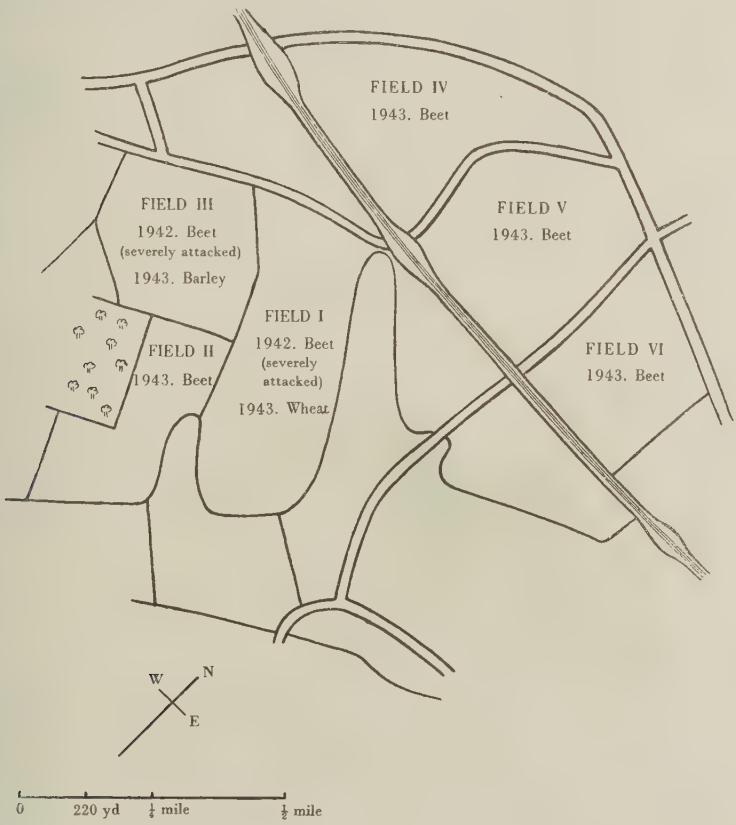

Fig. 1. Sketch-map showing position and cropping of fields under observation.

Oviposition of the third generation was first noted on 12 Aug. and appeared to be developing with great intensity on 19 Aug.; some beet were observed on that date carrying as many as 300 eggs. By 15 Sept. this last generation had completed its larval period. Pupae were collected for determination of emergence dates for spring 1944.

The dates for the three generations agree very closely with those found by Mayne & Van den Bruel

(1933). These workers also note that as some pupae of the second generation overwinter the injury by the third generation is less serious.

Oviposition of first generation—egg counts. In order to study the oviposition of the first generation of same position but it was not the same yard of drill row. The counting unit in all cases was 1 yard of drill row and 20 unit yards were taken on each count. At first, cotyledons only were available for egg-laying and numbers of eggs hatched and unhatched were

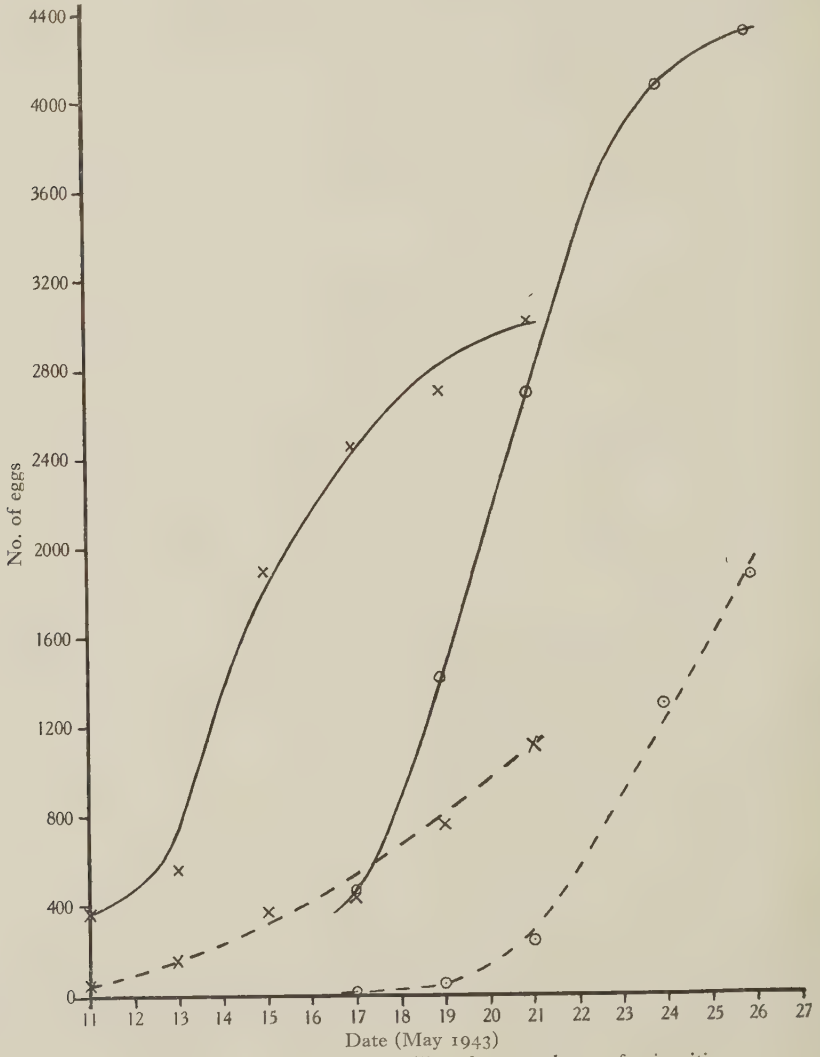

Fig. 2. Graphic summary of fertility of eggs and rate of oviposition.
——— total eggs. ----- hatched eggs. × cotyledon leaves. ⊙ true leaves.

Pegomyia egg counts were made at intervals by the following method. A starting-point was chosen at random about one-fifth across the width of the field. Subsequent sampling points were determined by taking 10 paces across the drill rows and 20 paces along. On each occasion when an egg count was made the starting-point was in approximately the counted, also the number of blistered leaves, dead cotyledons and dead plants. Later, as true leaves developed, the count was divided into two parts—cotyledons and true leaves. Immediately it became impossible to count satisfactorily on cotyledons owing to dying, etc., this part was abandoned. (Oviposition on cotyledons appeared to have ceased by

then—see Table 1, 21 May.) Counting on the true leaves continued until the crop was singled.

Hatched eggs were readily noted, as the egg-shell invariably persists on the leaf for a considerable time. This fact greatly facilitated accurate counts. Individual blisters could not be recorded satisfactorily as they tended to coalesce. Many small leaves contained as many as five larvae; therefore, blistered leaves were counted. These counts were interrupted owing to the chopping out of the beet on 27 May.

From Table 1 and Fig. 2 showing the rate of oviposition and hatching on cotyledons and true leaves, the following deductions may be made:

(1) Oviposition on the cotyledons was at a maximum on 14–15 May and ceased about 22–23 May.

TABLE 1. *Summary of egg-counts made on Fields II and IV*

Date	Total eggs: Not hatched	Total eggs: Hatched	Total eggs	Cotyledons: Blistered	Cotyledons: Dead
		Cotyledons. Field II			
11 May	325 (85·9%)	53 (14·1%)	378	—	—
13 ,,	441 (72·8%)	165 (27·2%)	606	—	—
15 ,,	1516 (79·8%)	384 (20·2%)	1900	118	—
17 ,,	2029 (82·4%)	434 (17·6%)	2463	201	—
19 ,,	1941 (71·9%)	757 (28·1%)	2698	310	62
21 ,,	1866 (61·9%)	1148 (38·1%)	3014	396	164
				True leaves	
		Cotyledons. Field IV			
26 May	408 (92·1%)	35 (7·9%)	443	11	—
28 ,,	353 (69·1%)	158 (30·9%)	511	121	—
2 June	130 (32·7%)	267 (67·3%)	397	201	—
		True leaves. Field II			
15 May	49	—	49	—	—
17 ,,	455 (99·3%)	3 (0·7%)	458	13	—
19 ,,	1405 (97·7%)	33 (2·3%)	1438	98	5
21 ,,	2442 (91·1%)	239 (8·9%)	2681	197	33
24 ,,	2766 (68·1%)	1294 (31·9%)	4060	532	144
26 ,,	2439 (56·7%)	1861 (43·3%)	4300	620	170

(2) Oviposition on the true leaves was at a maximum on 20–21 May and was much reduced by 26 May.

(3) Low fertility of the early eggs is indicated by the way in which the graph of hatched eggs on the cotyledons (first oviposition) lags behind the graph of hatched eggs on the true leaves (later oviposition) (see Fig. 2). Had the fertility of all the eggs been equal, these two graphs should have run parallel. This is further illustrated by the wide divergence between the graphs of the total and hatched eggs on the cotyledons, compared with the parallel nature of the graphs of total and hatched eggs on the true leaves. This latter indicates an almost 100% hatch.

Method of oviposition. In the initial stages of oviposition of the first generation it was observed that *Pegomyia* was depositing eggs singly, or occasionally two together, on each cotyledon. At the same time in an adjoining field, old beet tops growing among the wheat were carrying groups of four, six and eight eggs. As the seedling plants grew, so the tendency increased for larger groups to be deposited. With later generations, large groups were deposited immediately oviposition commenced, up to twenty being recorded. This indicates that the female is aware of the limited capacity of the cotyledons to provide food for the larvae and adjusts the egg-laying according to surface area of the leaf.

Migration of larvae. The movement of larvae from exhausted to fresh leaves was observed and is also illustrated by figures included in Table 1. On 17 May, with only three hatched eggs on the true leaves, thirteen were blistered. Again, on 19 May with thirty-three hatched eggs, ninety-eight leaves were noted to be blistered. This could only be accounted for by the migration of larvae from the cotyledons to the true leaves. On 21 May, a well-fed larva was seen to leave a cotyledon that was completely eaten away and enter a true leaf near the tip and proceed to form another blister; the time taken was 15 min. Blunck *et al.* (1933) note that a larva may leave its mine and seek another suitable place. The maximum distance a larva can travel on the ground seeking new plants is usually about 4 in.

Pupation. When the larvae were fully fed they dropped from the leaf and pupated at a depth of 2–3 in. in the soil immediately below the plant. With the first generation the pupae were all close to the tap root, and with later generations, on the circumference of a circle with the plant as centre and radius depending upon the size of the plant.

Severity of attack due to first generation. By the time egg counts ended, the average number of eggs per yard of drill row had reached 366. Over the whole field (27 acres) this gave an oviposition of almost 72,000,000 eggs. Imms (1942) states that there is parity of the sexes in *Pegomyia* and each female lays up to a maximum of fifty eggs. A simple calculation

gives the number of *Pegomyia* in the first generation as over 2,750,000. This must be a conservative estimate as it is highly improbable that all females laid fifty eggs. The number of eggs per plant, based on the above counts, was 8–9. The loss of plants from this attack was quite small. It may be deduced, therefore, that sugar beet even in the seedling stage can suffer a very heavy attack from *Pegomyia* without complete failure. However, the setback to the plants has serious results and reduces the final weight of the crop considerably.

Control

I. *Spraying*

Blunck (1927) first used the bait spray containing sodium fluoride in July and August 1926 and found it very effective. Bremer & Kaufmann (1927) also used it in 1926 in the same way. They used a 0·4% solution of sodium fluoride containing 2·5% of raw sugar. Molasses was said to be satisfactory as a sweetener. Decoux & Roland (1933) state that the most favourable time to destroy the flies is at the emergence of the first generation. The best results are likely to be obtained by spraying the cereals that have followed the beet crop with a sweetened solution of sodium fluoride. The edges of the grain fields and grass-grown ditches surrounding beet fields should also be sprayed, as the flies of the first generation frequent them in the intervals of oviposition. Flies of the later generations, on the contrary, remain entirely in the beet fields. Mayne & Van den Bruel (1933) found the 0·4% spray very effective when applied at midday in fine weather: the addition of 2% skimmed milk increased the wetting power and 2% sugar its attractiveness. 4% molasses gave better results apparently owing to its greater wetting power. They also found higher concentrations of sodium fluoride to be repellent.

Experiments with bait sprays containing sodium fluoride were carried out at Clipstone against the first and second generation. Field I, that had carried a crop of sugar beet in 1942 and was in wheat 1943, was known to have been heavily attacked by *Pegomyia* in 1942. Pupae had been collected from this field and retained out-of-doors to serve as a guide to the date of emergence in the field. Unfortunately, emergences were abnormally early and work on control began much sooner than expected. Flies were very numerous in the wheat field and spraying was commenced on 3 May. The flies were noted to be in great numbers around the margin of the field; it was, therefore, decided to spray a belt around the field to see if (*a*) the flies would take the poisoned bait and (*b*) if the poisoned bait had any marked killing effect.

The first spray used was essentially that recommended for trial by the Ministry of Agriculture and Fisheries (1942), beet molasses being used instead of sugar. The components were: sodium fluoride, 4 lb.; molasses, 9 lb.; water, 10 gal. The rate of spraying for this trial was 50 gal./acre. This seemed to give a more than adequate cover on the wheat and subsequently the recommended 25 gal./acre was used. The spray was applied by means of a Pierce Charlock Sprayer, and the reduction in quantity per acre was obtained by blocking alternate nozzles on the sprayer. The effect of the spray on the wheat was to produce a slight blackening of the flag, which later gave place to a yellowing effect, but there was no damage to the plant and eventually it was noticeable that there was, if anything, a finer plant as though an extra nitrogenous dressing had been given. This solution had a definite attraction for the flies and they were observed taking the bait readily.

On 6 May the field was visited and the molasses solution still persisted on the flag in small quantities. On this date a further experimental spraying was undertaken, using sugar instead of molasses. The following proportions were used: sodium fluoride, 4 lb.; sugar, 3 lb.; water, 10 gal. This was applied at the rate of 25 gal./acre. It was noticeable that the solution had little or no attractive smell, no flies were observed taking it and within ½ hr. a white crystalline covering was left on the flag.

On 6 May, 3 days after the first spraying, there was a large number of dead insects on the ground among the wheat; these included many *Pegomyia*. It appeared that the kill had been considerable, although no accurate measurement could be obtained to assess the result. After the second spraying there was little evidence that any further kill had been effected by the sugar/sodium fluoride solution.

Comparing the two sprays, it was clear that the molasses/sodium fluoride spray was far more efficient in every way than the sugar mixture.

Spraying against the second generation. Against the second generation, the 1943 beet field (Field II) was sprayed on 29 June and again on 6 July when the adults were numerous in the field. Part of the field (10 acres) was quartered and the two opposite quarters were sprayed. The mixture used was molasses/sodium fluoride at 25 gal./acre. There was no harmful effect on the young beet plants. Many dead flies were noted about the field 2 days after the spraying but, unfortunately, oviposition had commenced before the first spraying and subsequent examination of the field revealed no marked difference in damage between the treated and untreated quarters.

Experimental trapping to indicate possible fluctuations in population. It was decided that some indication of the results of the spraying might be obtained by means of trapping on the two fields known to have been attacked by *Pegomyia* in 1942, only one of which had been sprayed.

Technique. The habit of *Pegomyia* to remain at rest on the ground suggested that traps placed at ground-level would serve as indicators of the popu-

lation present. Traps were constructed of fibre-board (6 in. squares), coated with Stictite and secured to a central peg. Some were placed at ground-level, others 6 in. from the ground. The former were found to be the more effective; hardly any *Pegomyia* were taken on the raised traps. At each examination, the flies were removed, recorded and the Stictite touched up to ensure the continued efficiency of the traps.

Comparison between Field I (*wheat*, 1943; *beet*, 1942) *and Field III* (*barley*, 1943; *beet*, 1942). Ten traps were placed in each of the above fields and records taken. The traps were placed in position on 11 May, following the spraying of Field I (6 May). Table 2 is a summary of the flies caught in Fields I, III and II.

Up to 17 May the number of *Pegomyia* trapped in Field III was greater than in Field I. The initial populations of these two fields were assumed to be comparable on visual estimation of the damage in 1942. It appeared, therefore, that the lower population on Field I was due to the spraying. Normally, it would be reasonable to expect a higher trapping result in Field I where the wheat plant provided shelter for the flies; the barley in Field III gave no cover as it was only just showing. The alteration in relative populations on 17 May may be due to loss of effect of the spraying and to the better cover in the wheat retaining the flies that emerged later. The total number of *Pegomyia* trapped in the barley (III) was greater than in the wheat (I) up to 24 May.

Ten traps placed in Field II (1943, beet) showed an increasing number of flies at the time (12–26 May) when the population was decreasing in the source fields (I and III).

Spraying in 1944. In this year Field II was in wheat, and Field I sugar beet. *Pegomyia* commenced to emerge on 12 Apr. from collected material kept out-of-doors. In the field it was first noted on 26 Apr. Twenty-five traps of the type described above were placed in the wheat field (II) to keep a check on the population fluctuations. The peak emergence in the field was about 18 May. Spraying would have commenced on 15 May but owing to the machine being incomplete it was not possible until 18 May. A Weeks Tub Sprayer was used with a capacity of 120 gal. The poison bait spray (sodium fluoride/molasses as in previous years) was applied at about 30 gal./acre. A 21 ft. strip was sprayed round the headland of Field II and another strip of the same width 4 yd. farther inside the field. Weather conditions were ideal—a moist, humid morning with sunshine later. The flies were active and were observed to take the bait. Another spraying was carried out on 22 May. There was a marked reduction in *Pegomyia* from 26 May and no more sprayings were necessary. On 30 May the parasite (*Opius* sp.) was observed swarming in the beet field in such numbers that average sweeps on 1 yd. of drill-row on the headland gave twenty-five parasites. On 16 June it was noted that the attack from the first generation was negligible compared with the same generation in 1943. Evidence of damage from the second generation was even less noticeable and no damage at all occurred from the third.

Table 2. *Flies caught in fields under observation*

Date (May)	Field I, sprayed		Field III, not sprayed		Field II, beet, 1943
	Pegomyia	Other Anthomyidae	*Pegomyia*	Other Anthomyidae	*Pegomyia*
13	17	24	27	30	20
15	2	5	9	0	28
17	17	31	10	15	32
19	12	13	5	15	12
24	3	18	1	19	13
26	5	82	0	18	18
28	—	—	—	—	10

II. *Observations on the effect of the date of drilling of sugar beet on infestation*

Observations in previous years indicated that a certain measure of control could be obtained by cultural methods. The farmer's experience had been that mid-May drilling of sugar beet missed the first attack, the plants developed quickly through the susceptible stage and produced satisfactory crops. Also, in his experience, mid-April drilling in a year when the attack was heavy showed such a marked reduction in yield due to the attack by *Pegomyia*, that a better economic return was secured by drilling in mid-May to obtain a full stand although the weight per acre of beet was reduced owing to the delayed drilling.

In 1943, Field II, the earliest drilled, suffered a rather heavy attack, the seedlings coming through just before oviposition began. Further, it was situated between the two sources of supply (Fields I and III). Fields IV, V and VI, drilled later, missed the attack from the first generation. It was seen subsequently during the season, that *Pegomyia* never seriously affected the beet in Fields IV, V and VI. This suggests that the first generation establishing itself on a crop is of prime importance. Further, it leads to the suggestion that in areas where this pest causes

serious losses a certain measure of control might be effected by delaying the date of drilling to ensure that oviposition is completed before the seedlings appear.

TABLE 3. *Drilling dates and yields of fields under observation in* 1943

Field	Drilled	Damage due to *Pegomyia*	Yields/ acre tons
II	Last week April	Considerable	8–9*
IV	14–15 May	Almost free	8–9
V	19 May	Almost free	8–9
VI	23 May	Almost free	8–9

* Mr Shaw Browne's estimate of yield, had the field escaped attack by *Pegomyia*, was 11–12 tons/acre.

III. *Effect of parasites*

The flies are very subject to attacks by parasites. Interactions of host and parasites are very dependent on climatic conditions. Such interactions have been extensively studied by Blunck *et al.* in Germany and by Rambousek & Neuwirth in Czechoslovakia. Kaufmann (1930) states that theoretically 95 % parasitism, independent of the number of pupae, is needed to ensure a decrease of population. A forecast of infestation is made on this basis and on climatic data. Rambousek & Neuwirth (1931) discuss the possibility of forecasting the severity of infestation the following year by investigating the rate of parasitism of the hibernating pupae in the autumn. They give (1932) the types of seasons favourable and unfavourable to the flies.

From material collected at Clipstone for each generation the following increase in parasitism was noticed:

Generation	% parasites emerging
1st generation, 1943	Negligible
2nd generation, 1943	5
3rd generation, 1943	20
1st generation, 1944	80
2nd generation, 1944	95–100
3rd generation, 1944*	—

* Five man-hours were spent searching for pupae but none were found, indicating the almost complete parasitism of the 2nd generation.

This rapid increase in the number of parasites may be due to natural causes or it may be due to the reduction in numbers of *Pegomyia* as a result of the spraying. It cannot be denied that spraying as carried out during these 2 years must have had an effect on the balance of *Pegomyia* and its parasites, but a combination of both factors may be the true solution.

In Spring 1945, attacks by *Pegomyia* in central Nottinghamshire were severe and well distributed. At Clipstone, however, the attack by the first generation was almost negligible, indicating that the high parasitism during 1944 was but a localised occurrence, adding greater weight to the suggestion that the NaF/molasses spray might have been influential in the reduction of *Pegomyia* through the agency of its parasites.

IV. *Tentative conclusions on methods of control*

In Nottinghamshire it seems desirable to collect puparia of the second and third generations regularly to obtain the degree of parasitism. In addition, the time of emergence in spring could be obtained. From these data a safe date for sowing the beet could be recommended, and the date of spraying against the first generation. In certain years this emergence data may indicate that spraying would be unnecessary.

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The occurrence of *Anguillulina dipsaci* (Kühn.) on weed hosts, including new host records in fields of oats affected by 'tulip-root'

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The examination of eighteen weeds in three fields of oats affected with 'tulip-root' is described. Cleavers, common chickweed, mouse-ear chickweed and sandwort, were found to be hosts of *A. dipsaci*, the last two weeds being new host records. Successful transferences of *A. dipsaci* have been made from chickweed to oats and from oats back to chickweed. Transference of *A. dipsaci* has also been made from cleavers and sandwort to oats.

Goodey (1933) has listed many weeds found to be hosts of *Anguillulina dipsaci*, including the common chickweed (*Stellaria media* Vill.). Johnson (1938), making observations on fields of oats badly attacked by *A. dipsaci*, again records it on chickweed and also on cleavers (*Galium Aparine* L.), thus adding a new host plant to the list. His observations indicated that cleavers was probably a host for the bean strain of the eelworm; and since the same weed carrying eelworm occurred both among infested oats and beans, he suggested that the oat strain and the bean strain might be related.

A severe case of 'tulip-root' in oats near Cullompton was investigated in May 1944. There were three fields of oats on the farm. Owing to the farm having changed hands recently, information on previous cropping was unfortunately scanty.

Field I. Four acres of winter oats. Previous cropping: 1943, barley; 1942, wheat; 1941, oats; 1940, seeds hay. There was no knowledge of any trouble with the 1941 oats, but apparently the clover of the seeds hay had failed.

In the current crop of winter oats, the 'tulip-root' was very severe and fairly evenly distributed over the field, the headlands showing the least infection. This field was very weedy.

Field II. About 1½ acres of winter oats, which had been wheat during the previous year. The attack of 'tulip-root' was patchy, being only severe in small local areas. Weeds were not plentiful.

Field III. About 6 acres of winter oats. The 'tulip-root' occurred in two very well-defined strips running across the field, the oats in the intervening strips being free of any symptoms of eelworm attack; an examination of oats in the good strips showed *A. dipsaci* to be present but much less numerous than in the bad strips. The plants were several times taller and quite normal in appearance. There was an even gradation from severe 'tulip-root' to healthy-looking plants through a distance of 2 ft. all along the edge where the good strip adjoined the bad strip.

The only definite information on previous cropping was that the strip of good oats nearest to the entrance to the field had grown potatoes in 1943 and 1942; potatoes had not been grown anywhere else in the field, but it was not known just what other crops had been present; there is a strong probability that the rest of the field was roots or kale. Between this good strip of oats which had grown potatoes twice previously, and the gate, lay the worst strip of 'tulip-root'. The field was fairly weedy, weeds having flourished most in the 'tulip-root' areas.

Examination of weeds for the presence of Anguillulina dipsaci

The oat seed used in all three fields was the same, and the varying degrees of attack by eelworm therefore suggested that weeds rather than the seed might have been responsible for infection of the oats.

Field I. Samples of the weeds were collected and examined in the laboratory by means of Baermann funnels. Of eighteen weed species from this field the following were found to be heavily infested:

Common chickweed (*Stellaria media* Vill.).

Field mouse-ear chickweed (*Cerastium arvense* L.).

Cleavers (*Galium Aparine* L.). Not present in the field amongst the oats, but very plentiful in the hedges around the field.* It did not exhibit the yellowing of the lower leaves recorded by Johnson (1938), and no differences could be detected between infected plants and those free from eelworm; eelworm could be found in large numbers in the lower internodes and heavily infected plants had sometimes grown 4 ft. or more in height. The oats in the headland area showed little or no signs of attack by *A. dipsaci*, though some apparently healthy plants examined later showed light infections.

The mouse-ear chickweed constitutes a new host record. Neither this plant nor common chickweed showed any symptoms, even when the plants were heavily infested. In both, the eelworms were confined very largely to the lower two or three internodes.

* Excellent slides of the eelworms *in situ* in the tissues of cleavers were obtained, using the stain Scarlet R (Goodey, 1937).

The following weeds were present in the field but found to be free from *A. dipsaci*:

Scarlet pimpernel (*Anagallis arvensis* L.). Not plentiful.

Field pansy (*Viola arvensis* Murr.). Plentiful.

Field forget-me-not (*Myosotis arvensis* Hill). Plentiful.

Goosefoot (or 'fat hen') (*Chenopodium album* L.). Plentiful.

Sheep's sorrel (*Rumex acetosella* L.). Fairly plentiful.

Sow thistle (*Sonchus oleraceus* L.). Plentiful.

Common fumitory (*Fumaria officinalis* L.). Plentiful.

Creeping thistle (*Carduus arvensis* Robs.). Occasional.

Shepherd's purse (*Capsella bursa-pastoris* Medik.). Plentiful.

Parsley piert (*Alchemilla arvensis* Scop.). Fairly plentiful.

Perennial ryegrass (*Lolium perenne* L.). Plentiful.

Annual meadow grass (*Poa annua* L.). Plentiful.

Common horse-tail (*Equisetum arvense* L.). Plentiful.

A few 'ground-keeper' potatoes were found, but showed no infection.

Crosswort (*Galium cruciata* Scop.) was not present in the field, but was growing in company with the infected cleavers in the hedge; it was free from eelworm.

Field II. A little common chickweed in this field was found to be infected, also occasional plants of mouse-ear chickweed. No cleavers was present in the field or in the hedge. Crosswort in the hedge was not infected.

Field III. Seedling cleavers plants, present in small numbers amongst the oats, were heavily infected with *A. dipsaci*.

Common chickweed and mouse-ear chickweed were also abundant and infected.

Thyme-leaved sandwort (*Arenaria serpyllifolia* L.) was fairly plentiful and was heavily infected; like the chickweeds, it showed no symptoms of attack and the eelworms were again found in the lower internodes. This species also constitutes a new host record for *A. dipsaci*.

Cross-infection experiments

The observations made on this farm clearly offered much scope for cross-infection experiments and a number of trials were carried out.

From common chickweed

The inoculum consisted of the lower internodes of infected common chickweed from Field I, cut into short lengths and dried for 3 days after gathering. The dried inoculum was thoroughly mixed with the top 2 in. of soil in the pots in which various plants were grown. The experiments were started on 26 May and allowed to proceed until 29 July.

No infection took place with onion, Cornish Marl clover or Majestic potatoes. The onions and clover were sown as seed; the potatoes were sprouted when planted. Wild white clover, transplanted as small seedlings, was also tested but did not become infected.

Small seedlings of common chickweed from a cinder path were examined and found to be clean; some were transplanted and readily became heavily infected.

Oats became heavily infected, but did not develop symptoms of 'tulip root', though they were of weakly growth; no tillering took place under pot conditions. Oats sown without inoculum remained free from infection.

From 'tulip-rooted' oats to chickweed and back to oats

Chickweed seedlings, from the clean source previously mentioned, were grown in contact with chopped, dried, 'tulip-rooted' oat plants. A very heavy infection rapidly developed. By 29 July the chickweed was very luxuriant and apparently quite healthy, despite this heavy infection.

On 11 August this infested chickweed was dried and the chopped lower internodes used as an inoculum for oats sown on that date. By 17 Aug. the oats were ½ in. high and showed a light infection; by 24 Aug. they were 4 in. high and carried a very heavy infection but showed no symptoms of the attack. On 29 Aug. the oats were flattened to simulate rolling and promote tillering. By 25 Sept. tillering had still failed to take place and still no symptoms of 'tulip-root' had appeared though the plants were now looking very unhealthy.

From onions

As a check on the failure to infest onions from chickweed, clean chickweed seedlings were grown in contact with dried material from 'bloated' onions, heavily infected with *A. dipsaci*; no infection of the chickweed took place.

From cleavers

Oats grown with infected material became attacked by eelworm but did not show any symptoms.

From sandwort

On 8 Sept. oats were sown in contact with chopped, dried, eelworm-infected sandwort from Field III. By 26 Sept. the oats were some 4 in. high and were lightly infected with *A. dipsaci*, a number of larval forms making their appearance.

Observations in Field III after harvest

On 5 Sept. the oats had been cut and carried some time, but the good and bad strips already referred to

in this field were easily seen owing to the different densities of stubble. Chickweed from both the good and poor strips was found by examination in the laboratory to be infected, and the infections appeared to be very similar in intensity. Sandwort was also equally infected in both strips, but not so heavily as the chickweed. Volunteer oats about 3 in. high were common all over the field and were lightly infected to about the same extent on good and bad areas.

Discussion

The results of the observations and experiments show the existence on this Cullompton farm of a strain of *A. dipsaci* interchangeable between common chickweed and oats. There is also a strain in cleavers and one in sandwort which can establish themselves in oats, though the reverse process has not yet been carried out experimentally. The existence of infected mouse-ear chickweed and sandwort points to the possibility of a strain interchangeable between all five host plants, though much more work is required before this can be established. Johnson's observations on *A. dipsaci* in oats and beans are of especial interest, and it is hoped to attempt to transfer this strain of *A. dipsaci* to beans. As far as is known there is no recent history of the growing of beans on this farm.

The good growth of oats following on two successive potato crops, as compared with the severe 'tulip-root' adjoining, in Field III raises many interesting points. It is likely that the cultivation of two potato crops would reduce the weed populations as compared with the rest of the field and thus decrease the intensity of the initial infection of the oats. The oats and weeds in this area were, however, infected, though more lightly than the oats adjoining; differences in soil fertility, perhaps as the result of residual manures applied to the potatoes, may in part be responsible.

The occurrence in various common weeds of a strain of *A. dipsaci* capable of infecting oats presents a difficult problem, especially so where cleavers is abundant, since this plant is readily transported from one field to another clinging to sheep or other animals or to the clothing of farm workers.

The author wishes to express his gratitude to Dr T. Goodey for the interest he has taken, for much helpful advice and for his kindness in reading through the manuscript.

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A simple automatic window-opening device for temperature control in greenhouses

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(With 3 Text-figures)

The apparatus described opens greenhouse windows within 2 min. of the closing of a thermostatically controlled electric circuit, and shuts them with a time lag of 12 min. after the circuit is broken. Power is consumed, during the time that the circuit is closed only, at about 250 W.; the apparatus can readily be extended to operate more windows or other similar devices and is easily adaptable to existing greenhouses.

In greenhouse experiments carried out during the summer the chief difficulty is to prevent the temperature from rising too high; this is particularly troublesome when a generally high level has to be maintained and constant supervision is not available. The apparatus described has been installed in the hot half (*H*) of the greenhouse used for onion experiments, such as those described previously (Heath, 1943), in which an average temperature of about 25°C. is desired. The house is of the three-quarter span type, facing south, and the portion (*H*) in question measures 14 × 9 ft. × 7½ ft. high at the ridge

Essentially, the scheme consists in balancing the load of the windows with a bucket, into which water is pumped continuously during the time the windows are required to be open. A small outlet in the bottom of the bucket allows it to empty when pumping ceases. The small electric motor driving the pump is which raises water at the rate of 2 gal./min. from the usual storage tank *T* (in this case sunk in the floor under the centre staging). The water is delivered to two cylindrical buckets, each suspended under the centre staging by the operating cords from a pair of ventilators *R*. The cords are led over pulleys and

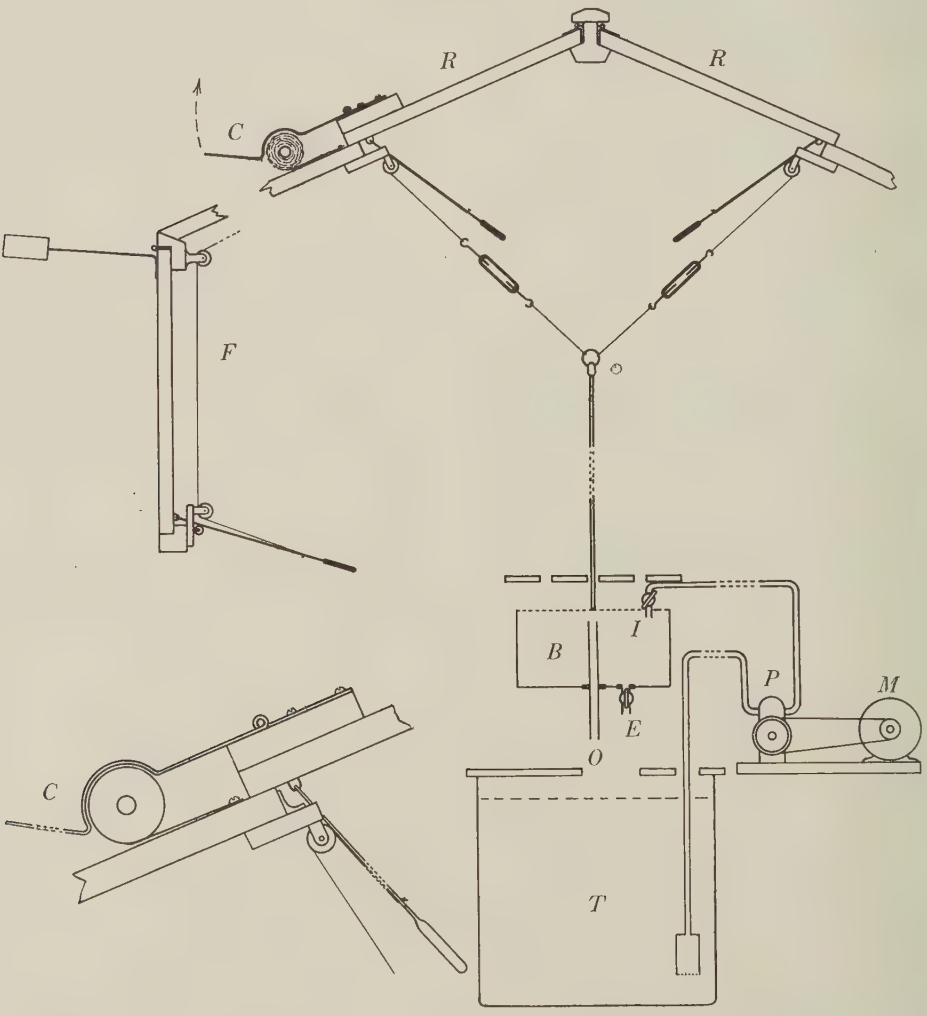

Fig. 1*a*. Enlarged detail of connexions to ventilator, and hinged catch for blind.

Fig. 1. Diagrammatic section of part of greenhouse showing automatic control for windows and blind. For explanation see text.

thermostatically controlled, and since an immediate response is not essential, large weights can be moved with a relatively small pump.

The diagram (Fig. 1) is almost self-explanatory. A bimetallic air thermostat working through a relay controls an $\frac{1}{8}$ h.p. electric motor *M*, switching it on at about 29°C. This drives a small gear-pump *P* connected directly to the buckets (see Figs. 1, 1*a*). Each bucket is fitted with an exit pipe *E* and an overflow *O*. These return the water to the tank either directly or through a collecting pan and pipe. As the rate of inflow at *I* from the pump is greater than the outflow from *E*, the water level in the buckets rises, ultimately opening the ventilators. These re-

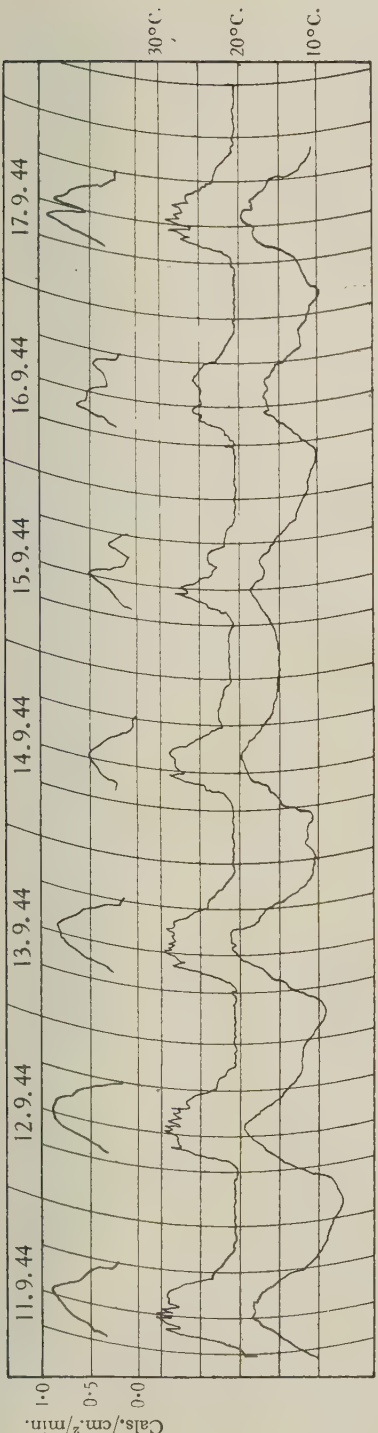

Fig. 2. Bottom graph: thermograph record of outside air temperature. Middle graph: the same near plants in house. Upper graph: total radiation.

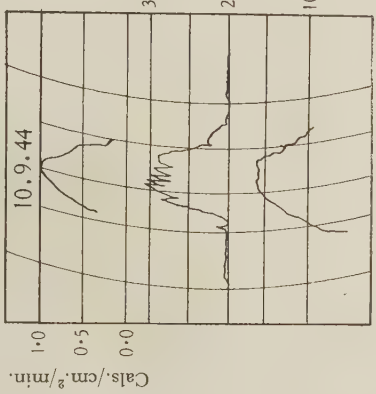

Fig. 3. As Fig. 2. A single day with bright sunshine

main open as long as pumping continues. When the temperature drops, the motor is switched off by the thermostat, the water level in the buckets falls and the ventilators close.

By means of taps on the inflow and exit pipes *I* and *E*, the rates of filling are so adjusted that one bucket operates an appreciable time before the other. The first bucket opens two roof ventilators *R* and also a front window *F*. The second operates the other two roof ventilators *R* and, by a simple hinged catch *C* (see Figs. 1, 1 *a*), releases the blind. The latter can only be raised again by hand. When this is done, the hinge allows the rolled-up blind to pass under *C*, which then holds it in position as long as the ventilator remains closed.

As at present adjusted, the first two ventilators and the front window open about 2 min. after the motor starts and the second pair of ventilators open and let the blind down some 10 min. later. After the motor stops, the second pair close within about 3 min. and the first pair together with the front window close after another 9 min. If outside conditions favour rapid cooling, however, only the first set of ventilators is opened before the motor stops, and the blind therefore remains up. The current consumed depends upon weather conditions, but since it is only of the order of 250 W. the cost cannot exceed a few pence per diem. In view of this, and the extreme simplicity of the components used, further complication whereby the motor is only run during actual opening or closing appears undesirable.

Results

In Fig. 2 the middle graph shows a week's thermograph record including both sunny and cloudy days, while in Fig. 3 is shown another record for a single day with bright sunshine. Between 7 p.m. and 6 a.m. G.M.T. the thermograph was moved with the plants into one of a set of electrically heated chambers, and this part of the chart is therefore irrelevant to the present subject. During the summer in daytime the temperature control is entirely effected by the automatic operation of the windows and blind, apart from a small (1 kw.) electric heater thermostatically controlled and switching on if the temperature falls to 20° C.; in winter, hot-water pipes are used also. The lowest graphs in Figs. 2 and 3 show the outside air temperature on the same scales, and the uppermost curves give the mean radiation in calories/cm.^{2}/min. estimated for each hour from the records of a Callendar Recorder. It will be seen that when insolation is sufficiently intense to raise the greenhouse temperature to about 27° C. the automatic control then maintains it between about 27 and 30° C. The initial ventilator opening when the blind is up occurs at a slightly lower reading on the thermograph, owing to the sun shining on the tin used to screen the thermostat. Since the plants are grown in similar tins and are also exposed to insolation when the blind is up, some such allowance for the effects of radiant heat appears desirable.

The steepness with which the graph frequently rises when the ventilators are shut and falls when they are open (Fig. 3) emphasizes the need for an automatic control of this nature. In full sunlight, with closed ventilators and the blind up, a temperature of 40° C. or more might well be reached in a short time, with disastrous results to the plants. With overcast sky and a wind blowing, the temperature would quickly fall to that of the outside air if the ventilators were open. The general practice in attempting to deal with this problem, in the absence of some such control mechanism, is to leave the ventilators open and the blind down and attempt to maintain the desired temperature by artificial heating. This is both extravagant in fuel and unsatisfactory in result. Although as here described the device is used mainly to prevent a hot-house from becoming too hot during the summer, it could similarly be used to prevent a cool house from becoming too cold in winter.

The authors wish to thank Mr W. Shaw, of the Research Institute of Plant Physiology, who set up most of the apparatus.

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Rhizomatous grass weeds and *Ophiobolus graminis* Sacc.

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An account is given of the infection of grass weeds by *Ophiobolus graminis* in a wheat crop affected by the disease. It was shown that the fungus was carried by the rhizomatous grasses, *Agropyron repens* and *Agrostis* spp. and also by *Holcus lanatus*. Examination of these showed the importance of *Agrostis* spp. in carrying over the mycelium under field conditions.

Many investigators have noted the presence of *Ophiobolus graminis* on grass weeds under field conditions and pointed out the importance of such weeds in perpetuating the disease either from original foci of infection in virgin grassland or from affected cereal crops. Experimental tests of the host range of *O. graminis* have been carried out by a number of workers and, while the criteria used differed, all were in agreement as to the importance of grass weeds as alternative hosts of the disease and as to the susceptibility of species of *Agropyron*, *Bromus* and *Hordeum*.

In a recent paper Buddin & Garrett (1944) reported cases of take-all in which they concluded that the occurrence of the disease in apparently satisfactory rotations was directly due to the presence of affected plants of *Agropyron repens*, *Agrostis* spp. and *Holcus lanatus*. They suggested that the effectiveness of such weeds in perpetuating the disease was due, not so much to their susceptibility to infection, as to their perennial habit and vigorous growth.

In 1944 an opportunity to investigate a similar case of take-all to those described by Buddin & Garrett occurred on one of the fields at the University Field Station, Warburton, Cheshire. The field was ploughed from old grass in the winter 1941–2 and in 1942 carried a series of silage mixtures in plots including lupins, rape, buckwheat, Italian ryegrass and oats. The ploughing and subsequent cultivation of the field did not completely destroy the original turf, and when the field was again ploughed many of the original sods were found undecomposed. In 1943 the whole area was cropped with potatoes followed by a series of wheat plots in 1944: in both years green shoots of couch grass were numerous after ploughing. The wheat plots were badly affected with *Ophiobolus graminis*, whiteheads being present throughout the crop while there was a number of patches where the plants were stunted and killed. While there did not appear to be any connexion between the disease in the wheat and the position of the silage plots containing Italian ryegrass or oats, the possibility that the outbreak might be due to *O. graminis* var. *Avenae* was borne in mind, but spore measurements proved that the wheat strain was responsible.

Table 1. *Grass species and infection by* Ophiobolus graminis *in samples of* 100 *plants*

	Agropyron repens		*Agrostis* spp.		*Holcus lanatus*		Other species		Total	
Sample no.	No. examined	No. infected	No. examined	No. infected	No. examined	No. infected	No. examined	No. infected	Examined	Infected
1	22	14	78	12	0	0	0	0	100	26
2	4	2	92	32	4	2	0	0	100	36
3	4	1	89	12	7	4	0	0	100	17
4	9	8	79	25	1	1	11	0	100	34
5	1	1	97	42	2	0	0	0	100	43
6	28	19	68	12	0	0	4	0	100	31
7	0	0	91	19	6	0	3	0	100	19
8	7	7	93	31	0	0	0	0	100	38
Total	75	52	687	185	20	7	18	0	800	244
Grasses present (%)	9·4		85·9		2·5		2·2		—	
Infection (%)	69		27		35		0		31	

Samples of 100 grass plants were taken at random from each of eight badly diseased patches, the number of plants of each species present was recorded, and the roots were washed free of soil and examined under the binocular dissecting microscope for lesions and discoloration of the vascular cylinder (see Table 1).

The examination of 800 plants collected at random indicated that the grass-weed flora of the affected

areas consisted mainly of *Agrostis* spp. (principally *A. alba* var. *stolonifera*) with an appreciable amount of *Agropyron repens* and some *Holcus lanatus*, with occasional plants of *Dactylis glomerata*, *Anthoxanthum odoratum* and *Poa annua*. *Ophiobolus* lesions were recorded on the three dominant species, the percentage infection being greatest on *Agropyron repens*.

To investigate more fully the infection of *A. repens* and *Agrostis* spp. a further examination of these two grasses was made. One hundred plants of each were collected at random from the affected patches; after washing the roots were examined for lesions under the binocular dissecting microscope and all affected plants discarded. Roots from the apparently clean plants were examined under the low-power microscope for runner hyphae (see Table 2). In both cases, while the percentage of plants heavily affected was substantially the same as in the first sampling, the further examination showed that there were some which, although apparently clean, were in fact bearing the mycelium, and that in the case of *Agrostis* spp. these lightly infected plants greatly increased the total.

TABLE 2. *Detailed examination of samples of* 100 *plants of* Agropyron repens *and* Agrostis *spp.*

	A. Plants heavily infected	B. Plants apparently clean: Runner hyphae present	B. Plants apparently clean: No runner hyphae present	Total no. of plants infected
Agropyron repens	71	11	18	82
Agrostis spp.	24	25	51	49

It was not possible to make isolates from the *Agrostis*, but spore measurements of ascospores obtained from perithecia from *Agropyron repens* agreed with those for *Ophiobolus graminis* and not with its variety *Avenae*.

The figures obtained show that *Agropyron repens*, *Agrostis* spp. and *Holcus lanatus* may all act as transmitters of *Ophiobolus graminis* and, in the case of *Agropyron repens* where the infection was severe and the rhizomes covered with plate mycelium, are in agreement with the experimental results of other workers (Kirby, 1922, 1925; Padwick, 1935). In the *Agrostis* spp. infection was much less severe and it was rare for lesions to occur on more than a few roots of affected plants, but the microscopic examination showed that many plants were bearing runner hyphae. In susceptibility tests Kirby (1922, 1925) reported that a number of species of *Agrostis* showed little or no infection under his experimental conditions, and subsequent workers agreed with his view that *Agrostis* spp. could be considered as resistant. Winter (1939) found that plants of *Agrostis spica-venti* which were apparently only slightly affected when examined 35 days after inoculation were dead, presumably through infection, after 5 months. Similar conflicting results were reported by Garrett (1941), who classed *Agrostis tenuis* and *A. alba* var. *stolonifera* as resistant on the basis of root discoloration but found that when a wheat seedling test was used for the estimation of survival both species of *Agrostis* were among the most effective propagators of the disease. The present observations show that under field conditions *Agrostis*, even though apparently unaffected, is, in fact, a host plant whose importance lies in its abundance and wide distribution.

These observations indicate that the *Ophiobolus* was carried over from the old turf by the rhizomatous grass weeds *Agropyron repens* and *Agrostis* spp. and also by *Holcus lanatus* although on account of the few plants present the latter was not important. They show the importance of such weeds as transmitters of infection and emphasize the need for adequate cultivation as a means of destroying them.

The author wishes to thank Dr J. H. Western for his advice and helpful criticism in the course of the investigation.

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Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 6 October 1944, at 11.30 a.m. at the London School of Hygiene and Tropical Medicine; the President, Prof. W. Brown, F.R.S., in the Chair.

The following papers were read and discussed:

Applied biology in Tasmania. By Dr J. W. Evans.
Applied entomology in South America. By Dr C. B. Williams.

Applied biology in Tasmania

By J. W. Evans, M.A., D.Sc., *Imperial Institute of Entomology, London, S.W. 7*

(*Abstract*)

The island of Tasmania, which has an area of 26,000 square miles and a population of 225,000 people, resembles England in several respects, possibly more closely than any other part of the British Empire. Some 300,000 acres are under cultivation, and the principal crops are deciduous fruits, hops, potatoes and wheat grown for biscuit flour. Sheep are raised in the central region, and there is an extensive dairying industry in the north.

The greater number of the insect pests has been introduced from abroad, but two native insects are particularly injurious. These are the light brown apple moth (*Tortrix postvittana* Walk.) and grass-grubs of various sorts.

The light brown apple moth has been introduced into England on more than one occasion, and is probably established here. The damage caused to apples by its caterpillars is similar to that of other Tortricid larvae and consists of surface blemishes on the fruit. There are two generations a year under orchard conditions, and the winter is passed in the larval stage. The larvae, which do not hibernate, are carried to the orchard floor when the leaves fall in the autumn, and remain there until the spring when they climb trees and complete their development on the opening buds. Only those larvae which have reached their third instar when the leaves fall are able to survive the winter months without feeding. This pest is difficult to control once larvae have reached the third instar as, thereafter, they tend to avoid feeding on poisoned foliage. However, larvae in earlier instars which occur in silken tunnels on the lower surface of apple leaves are readily destroyed by lead arsenate sprays.

'Grass-grubs' is a term applied in Tasmania to several distinct groups of insect larvae. The more important of these are the caterpillars of Hepialid moths belonging to the genus *Oncopera*, and the larvae of pasture dung beetles (*Aphodius* spp.).

Three species of *Oncopera* occur in Tasmania, and the most injurious species, *O. intricata* Walk., is confined to the State. Eggs are laid in February (late summer) and hatch after an incubation period of approximately 2 months. They are usually deposited under cover of some sort and fail to hatch if adequate moisture is not available in their immediate environment. At first the caterpillars feed close to the surface. Later they make deep vertical burrows from which they emerge at night-time to feed on the surface vegetation. They are not root-feeders. While the larvae of *Oncopera* are primarily pasture pests and will often entirely strip a pasture of all vegetation, they also, on occasion, injure certain crops. Control in pastures can be achieved by pasture management methods and on lawns by spraying with lead arsenate.

Aphodius larvae likewise construct vertical tunnels in the soil and feed at night on the leaves of grasses and clovers and also on dung. During July (mid-winter) pastures are frequently denuded of all vegetation by these insects.

Of the introduced insect pests, the lucerne flea (*Smynthurus viridis* L.) is of interest, since its importance as a pest in Tasmania is due to the occurrence of favourable climatic conditions rather than as a result of a release from the action of controlling organisms. This Collembolan, which is especially injurious to subterranean clover, is restricted to those areas of the State having a winter rainfall accompanied by moderate winter temperatures. The summer is passed in the egg stage, and the eggs hatch in the autumn. Breeding then continues until

early summer, when increasing dryness on the soil surface prevents further development of the eggs. Control is possible by spraying with lime sulphur (1–40) and by pasture management methods.

Four introduced insects which have been abundant in Tasmania because of the lack of parasites are the apple leaf-hopper (*Typhlocyba froggatti* Baker), the small cabbage white butterfly (*Pieris rapae* L.), the golden oak scale (*Asterolecanium variolosum* Ratz.), and the oak aphis (*Myzocallis annulata* Hartig).

The apple leaf-hopper, which causes a yellowing of apple leaves and specking of the fruit, is at present controlled to some extent following the introduction of a Mymarid egg parasite (*Anagrus armatus* Ashm.) from New Zealand.

The white butterfly, which was introduced into Tasmania as recently as 1940, is still present in injurious numbers, though a pupal parasite (*Pteromalus puparum* L.), introduced from New Zealand in 1942 and distributed in 1943, has become well established.

The oak scale, which until recent years was seriously affecting the health of oak trees, is completely controlled by a parasite (*Habrolepis dalmanii* Westwood), introduced from New Zealand in 1931. No parasites of the oak aphis have ever intentionally been introduced into Tasmania, but two occur in the State (*Aphelinus flavus* Nees and *Trioxys aceris* Hal.). These have been distributed artificially with beneficial results.

Of the numerous injurious mites established in Tasmania, two, the red-legged earth mite (*Halotydaeus destructor* Tucker) and the pea mite (*Penthaleus major* Dugès), are of interest as they furnish examples of control by cultural methods. The former is a pest of subterranean clover in pastures, and both mites are sometimes injurious to winter crops when such crops are planted on land previously under pasture. Infestations can be prevented if a well-kept autumn or early summer fallow is maintained. Thus, either the mites hatch when no food is available and hence die of starvation, or else conditions are rendered unsuitable for the deposition of over-summering eggs.

In conclusion, mention was made of one weed-control project, gorse and the gorse-seed weevil (*Apion ulicis* Forst.), and two examples were given of the difficulties of Plant Quarantine administration.

Applied entomology in South America

By C. B. Williams, M.A., Sc.D., *Rothamsted Experimental Station, Harpenden, Herts*

(*Abstract*)

Dr Williams gave an account of his recent visit to South America on behalf of The British Council. The object of the visit was to make contact with research workers in agriculture, and particularly in agricultural entomology in the different republics of South America; to see what were their problems; to see what help in solving these could be given from experience in the British Empire; and to put individual workers there in touch with workers on similar problems in Great Britain.

The visits to any one locality were of very short duration and in six months Brazil, Uruguay, Argentina, Chile, Peru, Ecuador, Colombia and Venezuela were visited in that order.

Photographs were shown of some of the principal agricultural research stations, universities and museums, and also of many of the scientific workers met on the tour.

Notes were given on various problems, including the following: locusts in Argentina and Ecuador; blackfly (Aleurodidae) on *Citrus* in Ecuador; smut (*Ustilago*) on sugar cane in Argentina; mosaic disease of sugar cane in many countries; a die-back disease of *Citrus* in Argentina and Brazil, believed to be due to incompatibility between stock and scion; froghoppers (Cercopidae) on sugar cane in Brazil and Ecuador; '*Bruchus*' on beans in Chile; pests of stored wheat in Brazil, Argentina and Chile; and of stored coffee in Colombia; fumigation methods in Argentina; pests of cotton in Argentina and Peru; thrips on wheat in Chile; antimalaria work in Ecuador; and blackberry as a pest in southern Chile.

The speaker explained that he had had no time either on the trip or during the lecture to go into details; but if anyone required further information on these points he would be glad to put them in touch with the workers in South America who were studying them.

Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 10 November 1944, in the Metallurgical Lecture Theatre of the Imperial College of Science and Technology, London; the President, Prof. W. Brown, F.R.S., in the Chair.

Discussion on nomenclature problems of the applied biologist

The following papers were read and discussed:

1. Reasons for name-change and the stability of names. By Dr J. Ramsbottom.
2. What are mycologists doing about nomenclature? By Dr G. R. Bisby.
3. Names of timber trees. By Mr B. J. Rendle.
4. How shall we name insects? By Mr A. Roebuck.
5. Synonymy of Aphididae. By Dr I. Thomas.
6. The International Code of Zoological Nomenclature. By Mr F. Hemming.

Reasons for name-change and the stability of names

By J. Ramsbottom, M.A., Dr.Sc., *British Museum (Natural History), Cromwell Road, London, S.W. 7*

One of the deep-seated fallacies amongst botanists in general, and academic botanists in particular, is that systematists have as their main object the upsetting of established names, particularly the time-honoured ones of text-books: doubtless zoologists have a similar strange belief in the superfluity of naughtiness of systematists. My part in this discussion is to state the problem.

We may assume at the outset that stability in nomenclature is a desideratum. How is it to be achieved? How would the non-systematist attain it? It may seem paradoxical but name-changes have as their object fixity and stability. What I assume is the main interest of economic biologists in nomenclature is to prevent name-changes which, even if sound in principle, are objectionable in practice.

Although the International Rules of Botany and of Zoology differ in several respects the general principles are the same. As I have a working experience only of the former my remarks will be confined to them.

The first set of *International Rules of Botanical Nomenclature* dates from 1905. Previously there were 'rules' of various kinds—and confusion. The *Rules* state: 'The essential points in nomenclature are: (1) to aim at fixity of names; (2) to avoid or to reject the use of forms and names which may cause error or ambiguity or throw science into confusion. Next in importance is the avoidance of all useless creation of names.' So as to get stability the idea of priority was adopted—the first validly published name is to be used. But if priority alone were to hold there would have been an appalling amount of name-changing as can be seen by glancing through the writings of Otto Kunze. Consequently, a starting date was fixed and, logically, Linnaeus's *Species Plantarum*, 1753, was chosen, for in it the binary system of nomenclature was first fully worked out and had almost immediately passed into common use. As Linnaeus had an autocratic way of using old generic names his errors of application, if there were such, have, of necessity, to be adopted. (Certain groups, Fungi in particular, had other starting points, similarly chosen with the idea of beginning with the work that was in general use. This was thought expedient so as to prevent legitimatizing names which were older and forgotten or neglected.)

But the drawing up of a set of International Rules was not beginning all over again: it was merely an attempt to straighten out an ever-increasing tangle. We can make Rules for the future: we cannot undo the past—we can say only that if such and such was done a name is legitimate, if such and such other conditions obtain it is valid and must be used.

Although we have got rid of all names published before 1753 many names were published afterwards in out-of-the-way journals and overlooked, and others were not taken up for various reasons which no scientific system of nomenclature could countenance. The *Rules* permit of conserving a generic name providing sufficient reasons are put forward for rejecting the older name. Though some systematists are opposed to this there is no doubt that it has been beneficial in retaining many well-established names which would otherwise have been replaced, some even by names which were literally revived.

At each International Botanical Congress there have been proposals to apply conservation to specific names, and they have always been rejected, often with not a single supporting vote.

If the idea really is to conserve specific *names* there is no possible chance of getting it through a Congress. If, however, it is to conserve specific epithets, the trivial name of Zoologists, but not of Linnaeus—it is a totally different matter. It will be better to return to this question after considering some of the ways in which changes of name, both generic and specific, arise.

Let us be perfectly clear about one thing. A too stable nomenclature means taxonomic stagnation, and without sound taxonomy we are building upon sand. The slogan 'One plant, one name', like many other slogans, shows little suggestion of intelligence. A plant can have as many names as genera in which it has been placed, and it may even have more than one specific epithet, as where the first one is already occupied in the genus to which a transference is made, or where the name of a new genus is that of a specific epithet—duplicating names being illegal in botanical nomenclature. Genera may be split up into smaller genera or may be combined into larger genera; or a species may be reduced in rank to that of a variety or vice versa.

Thus *Clitocybe tabescens* is often regarded as a ringless variety of *Armillaria mellea*; *Armillaria* has been subdivided and *A. mellea* placed in a separate genus *Armillariella*. *Clitocybe tabescens*, *Armillaria mellea* var. *tabescens*, and *Armillariella mellea* var. *tabescens* are all perfectly good names for the same fungus. An example of the lumping together of several genera is Quélet's genus *Rhodophyllus* recently adopted by Lange, which comprises the old *Entoloma*, *Claudopus*, *Nolanea*, *Lepiota* and *Eccilia*. If we regard this as taxonomically sound we have *Rhodophyllus lividus* for *Entoloma lividum*, *Rhodophyllus variabilis* for *Claudopus variabilis*, *Rhodophyllus pascuus* for *Nolanea pascua*, and many other disturbing name-changes. No standard list of names could prevent name-changes of this kind.

There is also misidentification. Sometimes it happens that a name in common use is found to be misapplied. This may occur only in those countries where a certain Flora is used; sometimes it is a gardeners' tradition. Such error leads to confusion, confusion made worse if the names are not changed.

Synonyms are another source of necessary change. We now know that *Boletus aurantiporus* is the same as *Boletus tridentinus*, and the latter must replace the former in our lists. A deeper consideration of synonyms would provide matters of a complexity not to be dismissed in a few words.

Just as it is illogical to use two or more names of the same kind for the same species, so is it to use the same name for different species, the so-called homonyms. Sometimes it is found that a much-used name is preoccupied by another species and a change must therefore be made. Also, as mentioned above, when a species is transferred to another genus it occasionally happens that the specific epithet is already in use in the genus and the new entrant has to be renamed.

There is also the *nomen nudum*, where there is no original description but only for a time a tradition with often a consequent rejection of a name which is exactly circumscribed. Obviously, certainty is preferable to a will o' the wisp.

So far as I can see none of the kinds of changes outlined above can be avoided. They are due to advancing knowledge—the segregation of new genera and the clearing up of confusion. They are taxonomic, not nomenclatural.

To a herbarium specialist working with tropical plants name-changes are of little consequence. He knows and appreciates the reasons for them and they do not incommode him in his work. But the matter is different when a whole literature has accumulated about a name. It is partly for this reason that I think a retrograde step has been taken by botanists who have decided that when a species is transferred from one genus to another the name of the author who makes the transference is appended whether he has had the correct species under consideration or not. (This cannot arise in zoology where only the name of the original author is cited.) Thus *Alternaria Cheiranthi* (Fr.) Bolle is taken to mean that Bolle decided, correctly, that *Macrosporium Cheiranthi* Fr. is an *Alternaria*. But it does not indicate that Bolle described and illustrated a different fungus under that name. Here we have an example of where the nomenclator is logical according to his lights. *Macrosporium Cheiranthi* Fr. is rightly an *Alternaria*, according to one view. Bolle realized this and made the transference.

The other view is that Bolle was concerned with the fungus she was describing and misidentified it with Fries's species. It is certainly disconcerting to a plant pathologist to find that there are two interpretations of *Alternaria Cheiranthi* (Fr.) Bolle, Bolle's own which is wrong, and that of orthodox mycological nomenclators. The decision concerning this interpretation was carried at Amsterdam and would have been reviewed at Stockholm in 1940. As the *Rules* stand it should still be open for reconsideration, and I think plant pathologists would be well advised to study the matter.

It has often been proposed to try to reach stability in nomenclature by having standard lists of conserved names. If this were done presumably each country would draw up such lists for each of the branches of economic botany, including teaching. We should eventually get a standard list of all names —a sort of dictionary. Such a standard list has been drawn up by some American horticulturists, but no systematist would regard it as satisfactory in any way.

One thing which might well be done would be to suppress names which have never been taken up, where their use would entail name-changes, especially of common organisms. There is no time to go into the arguments against this view—there are sound arguments but not weighty ones. The trouble is that frequently one name-change brings about others.

Some years ago I found a generic name in an obscure Italian medical paper which was prior to *Cicinnobolus*. At some time or other I should have recorded this and proposed that *Cicinnobolus* be conserved. But a species was described figured and christened. If I reveal the generic name to suppress it the specific epithet remains and a new combination will sooner or later result. Personally I should welcome a method of accomplishing legally what has to be done by the subterfuge of the blind eye—'If a room has a window there's no obligation to look out.' All black-outs, even self-imposed ones, are objectionable.

What are mycologists doing about nomenclature?

By G. R. Bisby, M.A., Ph.D., *Imperial Mycological Institute, Kew, Surrey*

Mycologists decided to follow the *International Rules of Botanical Nomenclature* adopted in 1905 and, before the following Congress in 1910, committees and individuals did effective work preparing the way for the Articles now numbered 20 (*e* and *f*) and 57. The second edition of the *Rules* in 1912 included these Articles, and fungi were also mentioned in three or four Recommendations or Examples. No generic name of a fungus was conserved, though 55 names of algae and 458 of phanerogams were.

Mycologists have made very little contribution to the *Rules* since 1910. Two Recommendations and a list of generic names proposed for conservation were added in 1930. No alteration relating exclusively to Fungi was passed at Amsterdam in 1935. The wording of Articles 20 (*f*) and 57 has proved to be susceptible of varied interpretation, but the 1910 words still stand. It seems evident that committees and individuals responsible for reporting to the next Congress should now be working on their projects.

However, mycologists have avoided nomenclatural confusion. Leading taxonomists and mycological societies have tried to follow the *Rules*. Certain 'unofficial' committees have done important work; for example, a Nomenclature Committee of the British Mycological Society reported on the credentials of various generic names proposed for conservation, and the Plant Pathology Committee has prepared lists of approved names of important pathogens. This *List of Common British Plant Diseases*, three editions of which have appeared during the last 15 years, have been very influential in stabilizing names—one of the objects of Rules of Nomenclature.

Since large numbers of mycologists are affected by the *Rules*, I venture to make a comment or two. First, the *Rules* are becoming rather lengthy. Excluding appendices, the second edition occupied nineteen pages, the third edition twenty-six, about three pages were added in 1935, and other additions have been proposed (e.g. in *Kew Bulletin*, 1939, pp. 317–26). It is evident that, if every mistake in nomenclature is to form the basis of an Article or Recommendation, the *Rules* must become longer and longer. Would it not be feasible to take a leaf from the Zoological Rules, and relegate items of very restricted application to a subsidiary and supplementary place? Thus, the addition to Article 61, still on trial, requires about 133 words (including examples) to tell us what to do if someone publishes, in one paper, the same name for two or more plants. A proposed addition of about the same length to Article 27 deals with Walter's use in 1788 of *Anonymos* as though it were a generic name. Secondly, a few entries in the *Rules* are worded somewhat ambiguously.

Since the *Rules* were prepared almost entirely by phanerogamic botanists for their own use, it is rather presumptuous for a mycologist to criticize them. Instead, the mycologists need criticism because of their neglect; for the nomenclature of fungi presents a number of special problems. Since there is the additional fact that parts of the botanical *Rules* (such as those applying to names of half-breeds and of plants of horticultural origin) do not affect mycologists, it would seem useful to have a special shorter code of nomenclature for Fungi, to apply under, and be supplementary to, the *International Rules*.

There are some problems of nomenclature which only the professional can decide; but there are others, such as the capitalization of epithets and the possible conservation of important ones, which the *users* of names might well consider. It should be possible to obtain the considered views of many plant pathologists, industrial mycologists, and others on such questions.

It is desirable that mycologists do more effective work on their nomenclature. The conservation of generic names should be considered further, so that the status of all important ones can be set forth prior to the next Congress, which can then conserve them. There is much more that committees or even indi-

viduals might do. It is obvious that the lead should be taken by the Special Committees appointed by Congresses; but, since these have accomplished so little during the last 35 years, it appears that some reconstruction of them is needed. Perhaps such committees should not be too large, some of the members younger, and inactivity of any member ought to result in his replacement.

Names of timber trees

By B. J. Rendle, B.Sc., A.R.C.S., *Forest Products Research Laboratory, Princes Risborough, Bucks*

I propose to say something about what has been done in recent years towards stabilizing the names of timber trees.

It is a long-standing complaint in forestry circles that fixity of names has not been achieved, and that as a result there has been a good deal of confusion in this particular branch of science. One of the principal reasons for this seems to be that it takes such a long time to ferret out all the evidence of priority and to get a definite ruling on each case. It seems that a good many of the names in general use 20–25 years ago were either incorrect at the time or have since been rendered incorrect by modifications of the *Rules*. The present period of transition during which grounds for changes in nomenclature are continually being brought to light happens to coincide with a period of unusual activity in economic botany. In the years immediately following the last war there was a revival of interest in forestry and timber research both in this country and in the Empire overseas. It was repeatedly happening that when the nomenclature of a timber tree was thoroughly investigated the name in general use was found to be wrong and a lot of well-known trees had their names changed. Then, in 1930, certain amendments were made to the *International Rules* which led to a fresh crop of name-changes.

Naturally, foresters found this sort of thing rather annoying, and some of them evidently decided that it wasn't good enough. The Forestry Commission quite early drew up an independent list of tree names for their own departmental purposes and still use these, I believe, in their official publications. A large proportion of their names are now incorrect but are perfectly understood by those concerned. The Commission must have saved their staff a good deal of trouble by ignoring the changes and counter-changes which have been taking place in the meantime.

In Australia, where they have suffered greatly at the hands of splitters and zealous researchers into early botanical records, it has been found expedient to fix certain well-known botanical names, sometimes in deliberate contravention of the *International Rules*. These have been published by the Standards Association of Australia in a list of Standard Reference Names of timbers for general use in the Dominion. For example:

Standard trade name	Standard trade reference name	Botanical name
River red gum	*Eucalyptus rostrata*	*Euc. camaldulensis* Dehn.
Southern blue gum	*Eucalyptus globulus*	*Euc. globulus* Labill. *Euc. bicostata* Maid., Blakely & Simmonds
Red tulip oak	*Tarrietia peralata*	*Argyrodendron peralata* Edlin

The idea is that the Standard Trade Reference Name shall be permanently retained for designating the commercial species and, in future editions of the list, any subsequent changes made by botanists shall merely be recorded in the third column. Very regrettable, no doubt, but it shows the sort of thing that is being done.

In some of the colonies the position has been complicated by the fact that trees have often been incorrectly determined owing to the difficulties of doing accurate systematic work under field conditions. Provisional determinations based on imperfect material have found their way into print, the names have become quite familiar and then, perhaps years later, have been shown to be incorrect. Then there is the modern tendency to split large genera into smaller units and to subdivide well-known species sometimes on what may appear to be insufficient grounds. I don't think taxonomists always pause to consider both sides of the question when they subdivide well-known economic species or raise varieties to specific rank or reduce species to varieties. If they did they might sometimes be forced to admit that the advantages of such changes to taxonomists may be outweighed by the disadvantages to economic botanists and others. It is not surprising that some economic botanists have the impression that taxonomists are more concerned with changing names than with stabilizing them.

The effects of some changes may be far-reaching. Economic plants are often cited by their botanical names in legislation, not to mention forest depart-

ment regulations, legal contracts for timber concessions, government specifications for timber, and so on. It is not difficult to imagine the confusion that could be caused (deliberately or otherwise) in a legal dispute hinging on the interpretation of a botanical name.

Foresters have been active in another direction. A constructive step towards the stabilizing of tree names was taken by the Third Empire Forestry Conference, 1928, when it was resolved to ask the Empire Forestry Association to compile a list of Standard Trade Names of Empire Timbers with their corresponding botanical names. This list was first published in 1930. It was revised and extended three times (in 1931, 1933 and 1938), each time with a number of changes in the botanical names so as to make these correct according to the current *International Rules*.

About this time the British Standards Institution was working on a series of standard specifications involving timber. They soon found the need of some reference list defining the identity of commercial timbers in terms of botanical species. They very wisely took the Empire Forestry Association list as a basis and extended this to include the numerous timbers from non-Empire sources which are marketed in this country. In 1935 a British Standard Nomenclature of Softwoods was published and in 1939 a similar list of Hardwoods. Quite recently the two lists have been thoroughly revised and combined in a single *British Standard Nomenclature of Commercial Timbers*, comprising something like 350 timbers, with the botanical species from which they are derived.

A typical entry is as follows:

Standard name	Botanical species	Other names
European oak	1. *Quercus robur* L.	1. *Q. pedunculata* Ehrh.
	2. *Quercus petraea* Liebl.	2. *Q. sessiliflora* Salisb.

I may say that it is extraordinarily useful to have an authoritative list of this kind for ready reference. It saves much time which would otherwise be spent in looking up names in different authorities and deciding which one to follow. At the Forest Products Research Laboratory, for instance, the names in the *British Standard Nomenclature* are regularly used in reports, publications and correspondence as well as for filing technical information on timbers and for cataloguing timber specimens. In fact, it is almost essential to have such a list for use not only by the handful of botanists on the staff but by other members of the technical staff including engineers, chemists and entomologists, and by clerks and typists as well. If this list or a similar one were not available I expect we should have compiled one for our own departmental use. If we had done so there would be a strong inclination to stick to it regardless of changes in orthodox botanical nomenclature.

As the names in the *British Standard Nomenclature* have been under continuous review now for about 15 years and the list has been continually checked and revised, it is probably as nearly correct as it can be.

A proposal to conserve specific names was submitted to the Sixth International Botanical Congress on behalf of thirty-eight forestry departments and other interested institutions. This was rejected, but as a compromise the Congress adopted Dr Ramsbottom's motion, 'That an International Committee be appointed to draw up a list of names of economic plants according to the *International Rules* and that this list may remain in use for a period of ten years.'

So far as I know very little publicity was given to this important decision of the Congress. It would be interesting to know how many of the economic botanists here have ever heard of it and how many have taken steps to ensure that the plants they are particularly interested in are being included in the list. I would suggest that anyone who has not already done so should take this opportunity of having the names of his plants checked by this Committee of experts. So far as timber names are concerned it is intended to have all those in the *British Standard Nomerclature* included in the International List of Economic Plants, subject to their being correct under the *International Rules*.

If the International Committee can finish its work within a reasonable period and produce a fairly comprehensive list of economic plants this will be so convenient that it will probably be used by a vast majority of economic botanists and will constitute a most important step forward in achieving fixity of botanical names.

How shall we name insects?

By A. Roebuck, *Midland Agricultural College, Sutton Bonington, Loughborough*

The first question which arises is why do we want a name? A name should be a designation by which a species may be distinguished so that one can write or talk about it without having always to present the specimens. Nowadays, it is sometimes better to carry the specimens about, and say, 'These are the ones referred to, you will know them by a variety of names.'

I believe the pioneers, such as Linnaeus, really gave names with this object in view, and that they had a genuine desire to help workers in the field. Nowadays, there seems to be no such desire, and what really happens too often is confusion by the rapidity with which names are changed.

To the worker in the field it is the insect that matters: a name is merely a name, although it is a very desirable working asset. To the nomenclaturist the name appears of paramount importance. There seem to be now two very distinct groups of workers unfortunately gradually becoming more detached. One group, the entomologists, are concerned with the living insects; the other group need never see them alive, and for long periods need never see them at all—their realm is literature.

Some of the latter appear to develop an irrepressible desire to find prior names and so upset established genera without regard to the confusion they produce. Then there are those who yearn to create new species; if allowed to do so there is nothing wonderful in that. I am more and more convinced of the truth of Lamarck's famous saying that 'a species is but a figment of the human imagination', and that 'in Nature individuals only exist'. There is thus an unlimited field for those who wish to create new genera and species, and it seems a pity that no sort of ordered procedure could be devised to avoid such a lot of the unnecessary difficulties which arise.

A very common pest has been placed in four different genera in the last few years. These different names have gone to farmers; it may be that all the names have gone to neighbouring farmers, or to fathers and sons. What is their reaction? Do they think they have different pests although they seem alike? Or, do they think we can't make up our minds what they have and make guesses? It is not a satisfactory position and we ought to avoid such situations if at all possible, and I believe it is possible. Such incidents do not add prestige to the scientists.

What is the position if we try to get the correct name? The nomenclaturists appear to be equally in difficulties. A short time ago I tried to name some species, in a difficult group, using a recently revised key. I seemed to be succeeding when I came to the following: 'It cannot be expected that I have used the correct names throughout.' This is very honest, but not exactly helpful. Later on, when the author of the key places three genera into one, he remarks: 'but when it even proves difficult to separate these insects into species, there seems no sufficient grounds for regarding them as belonging to different genera'. That is good; may others follow such an example. Surely such situations ought not to have arisen, but having arisen ought to be corrected as soon as possible. In 1933 a Committee on Generic Nomenclature was formed with limited functions. Only a small fraction of the work has yet been completed, or at any rate published. What has been published appears to me to offer little hope of full international agreement, while imposing sacrifices on our own entomologists. Still it is a step in the right direction.

Owing to international rivalries there seems to be little prospect of achieving any sort of stability for generations to come. I would suggest, therefore, that in the first instance the problem should be dealt with on a national or language basis. For much of our work it would not be necessary to use an international language. I would suggest English names for British insects. All the common ones and those of economic importance could easily be dealt with and others added as they became better known. This, I feel sure, would lead to a much greater interest being taken in them and would lead to an infinitely more stable nomenclature. The periodic attempts to interfere with common names by those with much enthusiasm but little experience should be discouraged.

We could then have the species listed with scientific names using generic and specific (trivial) names to which British entomologists have become accustomed. These lists should be supervised by some committee of the Royal Entomological Society and should have reasonable stability.

There would then be a third unstable list purporting to show the true position of our species in the world. This list would be complicated and very unstable. Possibly the Imperial Institute of Entomology or the committee of the Royal Entomological Society might supervise such a list.

For economic and popular bulletins the English names would be sufficient. A footnote could give the scientific name on the British list. For scientific work our British list could be used, with or without the common names. Should one wish to comment on, or study the systematic position of a species it should be possible to obtain initial guidance from the international list.

Very gradually the position of insects on the international list will become stabilized. Periodically, not necessarily at stated intervals, it will be necessary to amend the British scientific list to include those species upon which international agreement has been reached. There are difficulties to be settled in the choice of English names, but they offer a stability which would be an asset and which has become impossible of achievement by present methods. The British scientific list could also have a surprisingly great degree of stability when one considers the procedure adopted for achieving international agreement.

Synonymy of Aphididae

By I. Thomas, Ph.D., *University College of North Wales, Memorial Buildings, Bangor*

It is only necessary to quote one example of what has recently happened in the family Aphididae to show what confusion can result from the strict application of the 'Law of Priority'.

According to Hille Ris Lambers* the potato aphis, *Macrosiphum solanifolii* (Ashmead, 1882), should now be designated *M. euphorbiae* (Thomas, 1878). Unfortunately, Theobald stated that the species *solanifolii* Ash. was identical with *gei* (Koch, 1855), and the latter name, as a result of the application by Theobald of the Law of Priority, became well known in the literature. In all, Ris Lambers gives a list of twenty-four synonyms for this aphis. If it were possible easily to have the rules of nomenclature suspended in this instance probably all economic entomologists would like to see the name *solanifolii* established. The synonymy of the species *euphorbiae* (Thomas), *cholodkovskyi* (Mord) and *gei* (Koch) is as follows:

Macrosiphum euphorbiae (Thomas, C.A. 1878). Potato aphis.
M. [*Aphis*] *lactucae* (Schrank, 1801) partim.
M. [*Aphis*] *ulmariae* (Walker, 1848) partim.
[*Siphonophora*] *fragariae* (Buckton, 1876) nec (Koch, 1855).
S. euphorbicola (Thomas, 1878).
S. asclepiadifolii (Thomas, 1879).
S. cucurbitae (Thomas, 1879).
S. tulipae (Monell, 1879).
S. citrifolii (Ashmead, 1880).
S. solanifolii (Ashmead, 1882).
[*Nectarophora*] *asclepiadis* (Cowen, 1895).
N. tabaci (Pergande, 1898).
N. citrifolii (Clarke, 1903).
N. lycopersici (Clarke, 1903).
Macrosiphum stellariae Theobald, 1912.
M. sileneum Theobald, 1913.
M. centranthi Theobald, 1915, partim.
M. hibernaculorum Theobald, 1916.
M. euphorbiellum Theobald, 1917.
M. epilobii Jackson, 1919.
Macrosiphum epilobiellum Theobald, 1923.
M. hellebori Theobald & Walton, 1923.
M. amygdaloides Theobald, 1925.
M. gei Theobald, 1926, partim.
M. cholodkovskyi Opmanis, 1928, partim.

Macrosiphum cholodkovskyi (Mordvilko, 1909). Host plant: *Ulmaria palustris* Mnch.
M. [*Aphis pisi*] (Kaltenbach, 1843), partim.
M. [*Aphis ulmariae*] (Walker, 1848), partim.
Macrosiphum gei Theobald, 1926, partim.

Macrosiphum gei (Koch, 1855). Host plants: species of *Geum*, *Chaerophyllum*, *Eryngium* and *Anthriscus*.
M. [*Aphis*] *pisi* (Kaltenbach, 1843), partim.
M. [*Aphis*] *ulmariae* (Walker, 1848), partim.
Macrosiphum corallinum Theobald, 1925.

As a result of a discussion on the Law of Priority the Royal Entomological Society set up a Committee on Generic Nomenclature, but their lists are sometimes not accepted even by the authors of the lists themselves—vide Balfour Browne.†

It has been suggested that economic entomologists should have a stabilized list of Latin names. This, however, would lead to there being an ever-widening gap between the economic entomologist and the systematist. It should, nevertheless, be possible to stabilize nomenclature for 10 or 20 years and then make the necessary changes at the end of such a period but not before asking the Zoological Commission to suspend the rules in cases where 'greater confusion than uniformity' would result from a change.

Entomological literature is still suffering from the effects of inadequate descriptions of proposed new species. Some of the descriptions given even in present-day literature are totally inadequate. All such work has to be sifted by the expert systematist, and it would considerably lighten his labours if something could be done to establish a minimum standard for publications.

* Hille Ris Lambers, D. (1939). *Temminckia*, **4**, 84.

† Balfour Browne, F. (1943). *Ent. Month. Mag.* Feb., p. 46.

The International Code of Zoological Nomenclature

By Francis Hemming, C.M.G., C.B.E., *Secretary to the International Commission on Zoological Nomenclature*

The International Code of Zoological Nomenclature adopted in 1901 by the Fifth International Congress of Zoology was drawn up primarily by workers in systematic zoology in order to meet the needs of workers in that field. This was both natural and inevitable: natural, because the first impact of nomenclatural questions has to be met by the systematist; inevitable, because the majority of the members of

the Congress were themselves workers in systematic zoology. Very great difficulties were encountered at Berlin in securing unanimity in regard to the proposed Code, and that unanimity was only achieved by placing a greater stress on the Law of Priority than had previously been intended.

The International Code won a rapid acceptance among systematic zoologists, but owing to the rigidity of Article 25 (Law of Priority) difficulties were soon encountered, since the application of that Article led to well-known and well-established generic names being replaced by older but less well-known names. Objection was taken to such changes, not only by numerous systematic zoologists, but also by workers in applied biology, who argued that, whatever rules of nomenclature systematists might adopt for their own purposes, those rules should be such as to secure a reasonable degree of stability for important names widely used in the applied field.

The International Congress tried to meet this criticism at their meeting held at Graz in 1910 in which they agreed in principle to establish an 'Official List of Generic Names in Zoology, with their type species'. It was hoped that in this way it would be demonstrated that the Berlin Code of 1901 would secure stability for a wide range of important names, and therefore that the area of disagreement would be greatly narrowed. In 1913 at Monaco, the International Congress again considered this problem and decided to settle it by the grant (subject to certain safeguards) of plenary powers to the International Commission on Zoological Nomenclature to suspend the rules as applied to any given case where, in the judgement of the Commission, the strict application of the rules would clearly result in greater confusion than uniformity. It will be seen, therefore, that, as regards zoological names of importance either to workers in the applied field or to workers in systematic zoology, two valuable and important instruments have been placed in the hands of the International Commission in order to assist in the stabilizing of zoological nomenclature.

The International Commission attach great importance to the *Official List* which they are most anxious to develop in order that as rapidly as possible it may contain the names of all the most important genera in each of the various Phyla and Classes of the Animal Kingdom. Arrangements are now being made for the early publication in book form of the *Official List* in which will be found full bibliographical particulars relating to the names (between 600 and 700 in number) which have so far been admitted to the *List*, and the International Commission are hopeful that the publication of the *Official List* in this way will provide a striking demonstration of the value of securing the admission of generic names to the *List* and will stimulate the submission by specialists of proposals for further additions to the *List*.

The International Commission consider that in the selection of names proposed for addition to the *Official List* attention should be paid not only to the importance of given generic names in systematic zoology, but also to the applied fields such as medicine, agriculture, veterinary science, stratigraphy and the other branches of science, workers in which have to use the scientific names of animals. The International Commission desire also to place on the *Official List* names commonly used in—and therefore of importance to—the teaching of zoology at the Universities. The International Commission cannot itself be expected as a body to select for admission to the *Official List* names which are of special importance from all the various points of view indicated above, and they desire, therefore, by all means in their power to enlist the help of workers in these fields. The Association of Applied Biologists can be assured that, where any group of workers notify the International Commission regarding names which they would like to see stabilized by admission to the *Official List*, the Commission, for its part, will do everything possible to meet their wishes in this matter.

Here and there cases will no doubt arise where it will be found that it is impossible to place some well-known name on the *Official List* without the Commission first suspending the rules in order to validate that name by suppressing some earlier name or by fixing as the type of the genus some species other than that which is the type under the International Code. Where there are such cases, the sooner they are brought out into the open and settled once and for all, the better for all concerned.

The International Commission on Zoological Nomenclature, acting for the International Congress of Zoology, fully appreciate the need for meeting the requirements of workers in the applied field; they are already doing what they can to assist such workers by overcoming difficulties of nomenclature; but their power to give practical assistance depends to a large extent upon the efforts which workers in the applied field are themselves willing to make in order to put the Commission in possession of the data necessary to enable them to take effective action.

Report of the Council of the Association of Applied Biologists for the year 1944

The Officers and Council of the Association were as follows:

President: Prof. W. Brown, D.Sc., F.R.S.

Vice-Presidents: E. W. Mason, M.A., H. W. Miles, D.Sc.

Hon. Treasurer: W. C. Moore, M.A.

Hon. Secretaries: General and Botanical: W. P. K. Findlay, D.Sc.; Zoological: H. F. Barnes, M.A., Ph.D.

Hon. Editors of Annals of Applied Biology: General and Botanical: Prof. W. B. Brierley, D.Sc.; Zoological: C. T. Gimingham, B.Sc., F.I.C.

Council: L. A. Allen, Ph.D., G. M. Bates, D.Sc., F. C. Bawden, M.A., J. R. Busvine, Ph.D., G. V. B. Herford, B.A., E. W. Mason, M.A., H. W. Miles, D.Sc., H. Shaw, Ph.D., W. Steer, M.A., I. Thomas, Ph.D., W. A. R. Dillon-Weston, M.A., Miss A. P. Wilson, A.R.C.S.

The Council are again able to record a year of successful progress for the Association. There were held in London six meetings, all of which were well attended, an average of 48 Members and 21 visitors recording their names at each meeting. The total membership now stands at 371, a net increase of 27 over the previous year. A total of 33 new Members was elected; this is the largest number of admissions in any year since 1920, and only four Members resigned. The Council has to record with great regret the deaths of two Past Presidents: Sir John B. Farmer who was President in 1918 and Sir David Prain who was President 1919–21 and took an active part in the reconstruction of the Association after the last war. Also of Prof. P. Marchal, one of the Honorary Members, Miss Dorothy Ashworth, and Mr L. C. Comrie.

The following papers were presented to the Association during the year:

18 Feb. Discussion, opened by Prof. W. B. Brierley, on the Organization of the Agricultural and Horticultural Advisory Services in Great Britain, with special reference to the recommendations in the Luxmoore report. Mr Ronald Ede, who had been Secretary of the Luxmoore Committee, outlined its proposals and discussed their implications.

19 Apr. Joint meeting with the Agricultural Group and the Microbiological Panel of the Food Group of the Society of Chemical Industry in the rooms of the Chemical Society. *Discussion on soil sterilization.* W. F. Bewley: Some problems in soil sterilization. W. J. C. Lawrence: Soil sterilization and seedling growth. A. H. Dodd: Considerations in chemical soil sterilization. H. Lees and J. H. Quastel: Demonstration of a new technique for the study of soil sterilization. Effects of chlorate administration on soil nitrification.

23 June. *Symposium on cereal diseases.* W. C. Moore: General introduction. Prof. F. T. Brooks: Biologic specialization in the rusts. W. A. R. Dillon-Weston: Seed-borne diseases. R. W. G. Dennis: Cereal diseases in Scotland. D. Walters Davies: Cereal diseases in mid-Wales. S. D. Garrett: 'Take-all' disease. Miss Glynne: Cercosporella and lodging. General discussion.

6 Oct. J. W. Evans: Applied biology in Tasmania. C. B. Williams: Applied entomology in South America.

10 Nov. *Discussion on nomenclature problems of the applied biologist.* J. Ramsbottom: Reasons for name-change and the stability of names. G. R. Bisby: What are mycologists doing about nomenclature? B. J. Rendle: Names of timber trees. A. Roebuck: How shall we name insects? I. Thomas: Synonymy of Aphididae. F. Hemming: The International Code of Zoological Nomenclature.

8 Dec. T. Goodey: Eelworm diseases of onions: some recent investigations. F. R. Petherbridge: The biology and control of the carrot fly. A. Roebuck: The carrot fly in the Midlands. G. Fox-Wilson: Investigations into the control of the carrot fly under garden conditions.

In order to express the appreciation of the Association for the long and valued services of the Honorary Editors, the Council unanimously agreed to vote honoraria to Prof. W. B. Brierley and Mr C. T. Gimingham. The latter resigned from the Zoological Editorship at the end of 1944 after nine years' service for which the Council wishes to express on behalf of the Association its grateful thanks.

The Council wish to express their appreciative thanks to the Authorities of the Imperial College of Science and Technology and of the London School of

Hygiene and Tropical Medicine for so kindly affording the Association accommodation for their meetings.

At an Extraordinary General Meeting, held on 10 Nov., the Laws governing the election of Council were altered by a unanimous vote so that, in future, nominations for Council should be invited from the general body of Members before the Council makes its nominations.

In view of the increasingly wide range of subjects now covered by the Association, many of which cannot be classified as strictly botanical or zoological, i.e. such subjects as microbiology, 'plant protection', agricultural statistics and so on, the Council decided that the time has come to cease dividing the Association into botanical and zoological sections with their appropriate Officers and to adopt instead the principle of affording representation of all the various interests on the Council, at the same time retaining a strong representation of entomology and plant pathology. The Hon. General Editor expressed the view that more efficient working will be secured by having all manuscripts submitted to him in the first instance. Papers on subjects with which he was not specially familiar will then be referred to the Assistant Editor, who would normally have a different range of interest to the General Editor, and to members of an active Publications Committee who would be chosen for their specialized knowledge as well as for their editorial capabilities.

In furtherance of the decision taken in 1943 and noted in the last Report of the Council, a Plant Pests and Diseases Committee was set up in February to advise the Council on matters concerning plant pests and diseases. The Rules laid down by the Council and governing the activities of the Committee provide that four members shall retire annually and shall not be eligible for immediate re-election, and that no member shall serve on the Committee for more than three consecutive years. Since the Committee had been in existence for less than 12 months at the end of 1944, it was decided that none of the members should retire that year.

The members nominated by the Council to serve on the Committee were: G. C. Ainsworth, Miss M. T. Franklin, S. D. Garrett, H. C. Gough, H. G. Kearns, W. G. Keyworth, J. T. Martin, M. H. Moore, C. Potter, I. Thomas, J. H. Western, Miss A. P. Wilson, and the President of the Association *ex-officio*.

Plant Pests and Diseases Committee: Report for 1944

At the first meeting of the Committee, J. T. Martin was elected Chairman and W. G. Keyworth Secretary.

Three meetings have been held and three matters of interest to plant pathologists have been selected for special consideration:

(1) A study is being made of the methods of recruitment and training of plant pathologists and of the facilities for research on plant pathology in Great Britain, with the object of submitting recommendations to Council for the development of plant pathology in this country.

(2) In collaboration with the Ministry of Agriculture Conference of Advisory Entomologists, a Sub-Committee has been formed to compile a list of Pests of British Plants. This very considerable task had already been started by the Conference of Advisory Entomologists and the work will now be continued by the Joint Sub-committee.

(3) The Director of the Imperial Mycological Institute asked the Committee for its opinion on the names of the British diseases included in the 'List of Virus Names used in the *Review of Applied Mycology*'. Four of the Association's leading virologists—F. C. Bawden, R. V. Harris, G. Samuel and K. M. Smith—were co-opted to a Sub-committee set up to undertake this work and a report has been submitted to the Imperial Mycological Institute.

Hon. Editors' Report for 1944

Vol. **31** of the *Annals of Applied Biology* comprised pp. viii + 387 and 11 Plates as against pp. viii + 400 and 10 Plates for vol. **30**. Including papers published in *Proceedings*, vol. **31** contained 63 papers, 54 being by Members of the Association. The subjects may be roughly classified as follows: general applied botany, 5; mycology and fungus disease, 16; viruses and virus diseases, 5; entomology and insect pests, 13; plant protection, insecticides and fungicides, 12; helminthology and nematode diseases, 2; applied microbiology, 2; general, technique and statistics, 5; obituary notices, 3.

Publication of the *Annals* was delayed owing to war-time production difficulties, and the several numbers of vol. **31** were issued on the following dates: No. 1, 12 Apr. 1944; No. 2, 26 July 1944; No. 3, 20 Oct. 1944; No. 4, 25 Jan. 1945.

Report of the Honorary Treasurer for the year ended 31 December 1944

During the year ended 31 Dec. 1944, the amount received for subscriptions and entrance fees, including arrears, was £441. 7*s*. 0*d*., which was £38. 12*s*. 6*d*. more than in 1943. There were only three subscriptions remaining unpaid at the end of the year.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £724. 17*s*. 6*d*., an increase of £52. 4*s*. 2*d*. The volume contained 387 pages compared with 400 pages in 1943, but owing to a further increase of 20% in the printing charges, the cost of producing it rose by £151. 17*s*. 11*d*. to £1346. 3*s*. 6*d*. There was a noticeable drop in the sales of back volumes and parts, which realized £77. 4*s*. 2*d*., as compared with £175. 8*s*. 3*d*. in 1943. The balance against the *Annals* on the year's working was £507. 5*s*. 4*d*.

Expenditure for the year exceeded income by £101. 5*s*. 7*d*., and in addition the Council unanimously voted a sum of £131. 5*s*. 0*d*. as honoraria to the Editors for their long and valued voluntary services to the Association. Most of this deficit of £232. 10*s*. 7*d*. was, however, made good by a grant of £200. 0*s*. 0*d*. allocated to the Association by the Council of the Royal Society from the special Rockefeller Foundation Grant in aid of Scientific Publications, a grant which the Council wish to acknowledge with gratitude. After all obligations had been met, the assets of the Association at the end of the year amounted to £1095. 11*s*. 4*d*., of which £735. 13*s*. 10*d*. was represented by National Savings Certificates, and £116. 3*s*. 0*d*. by the estimated value of the stock of the *Annals*.

W. C. MOORE
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1944 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.
To Estimated Value of Stock, 1 January 1944	115	11	0	By Sales—Current Volume	724	17	6
To Cambridge University Press	1346	3	6	By Sales—Back Volumes, Parts and Sets	77	4	2
				By Sales of Reprints and Advertisements	36	4	6
				By Estimated Value of Stock, 31 December 1944	116	3	0
				By Balance, carried down	507	5	4
	£1461	14	6		£1461	14	6

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1944 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.	£	s.	d.
To *Annals of Applied Biology*, balance brought down	507	5	4	By Members' Subscriptions:						
To Printing and Stationery	10	13	7	Arrears	21	5	0			
To Postages and Cheque Stamps	14	3	9	Entrance Fees	12	12	0			
To Subscription—Parliamentary Science Committee	10	10	0	Current	407	10	0			
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	20	17	11					441	7	0
To Expenses of Plant Pests and Diseases Committee	7	15	7	By Interest Receivable:						
To Audit Fee Reserve	5	5	0	National Savings Certificates	20	0	6			
				Post Office Savings Bank Account	13	17	10			
				Bank Deposit Account			3			
								33	18	7
				By Balance, being Excess of Expenditure over Income for the Year				101	5	7
	£576	11	2					£576	11	2

BALANCE SHEET, 31 DECEMBER 1944

Liabilities and Surplus	£	s.	d.	£	s.	d.	£	s.	d.	Assets	£	s.	d.	£	s.	d.
Sundry Creditors:										Cash at Bank:						
Cambridge University Press				562	17	4				Current Account	246	15	10			
Audit Fee Reserve				5	5	0				Post Office Savings Bank Account	658	11	7			
Sundry Expenses				23	9	7								905	7	5
							591	11	11	500 National Savings Certificates				735	13	10
Subscriptions and Entrance Fees, paid in advance							45	1	0	Stock of *Annals of Applied Biology* at estimated value				116	3	0
Life Membership Fund Reserve							25	0	0							
Excess of Assets over Liabilities:																
As per Balance Sheet of 31 December 1943				1128	1	11										
Add: Rockefeller Foundation Grant (per Royal Society)				200	0	0										
				1328	1	11										
Less: Honoraria to Hon. Editors	131	5	0													
Balance of Income and Expenditure Account for 1944	101	5	7													
				232	10	7										
							1095	11	4							
							£1757	4	3					£1757	4	3

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed) H. J. COX & CO. *Incorporated Accountants* } *Auditors*

HARPENDEN, 20 *February* 1945